ASSESSING THE IMPACT OF THE KAPAHULU STORM DRAIN SYSTEM ON THE QUALITY OF WATER AT KUHIO BEACH AND THE HEALTH OF THE SWIMMERS USING THE BEACH

Project Completion Report

March 1994

PREPARED FOR
State of Hawaii
Department of Health
Contract No.: ASO Log No. 92-613
Project Period: 1 April 1992-31 December 1993

Principal Investigators:
Roger S. Fujioka and David M. Morens

WATER RESOURCES RESEARCH CENTER
University of Hawaii at Manoa
Honolulu, Hawaii 96822

EXECUTIVE SUMMARY

Kuhio Beach is one of the most popular beaches in Hawaii for at least three reasons. First, it is located in Waikiki where tourists concentrate. Second, the beach is very visible and accessible. There are no hotels fronting the beach and one can easily walk from Kalakaua Avenue onto the sandy area and into the water. This beach site is especially accessible to people who are not staying in the few hotels lining the rest of Waikiki Beach. Third, the water at this beach is always calm as this beach is essentially closed by walls. As a result, this beach is especially desirable for children and for people who are not strong swimmers. The water at this beach site has been designated for recreational use and the State of Hawaii has been monitoring the quality of water at this beach. Beginning in the early 1970s and throughout the 1980s, the recreational water quality standard established by the USEPA was a geometric mean of 200 fecal coliform/100ml. During this time period, the waters at Kuhio Beach consistently met this water quality standard.

During the 1970s, the USEPA completed an extensive epidemiological and water quality study conducted at New York (Coney Island Beach, Rockaway Beach), Massachusetts (Boston Harbor Beach) and New Orleans (Lake Ponchatrain). Based on the results of that study, USEPA concluded that concentrations of enterococci bacteria but not fecal coliforms in marine waters could be related to incidences of diarrheal diseases among swimmers. As a result, USEPA recommended that all states change their marine recreational water quality standard to a geometric mean of 35 enterococci/100ml. In 1990, the State of Hawaii accepted the recommendation by the USEPA but set a more stringent standard of a geometric mean of 7 enterococci/100ml. After this new standard was established, the results of the State of Hawaii water quality monitoring program showed that the water at Kuhio Beach could no longer consistently meet this new water quality standard. This raised the question as to the source of the enterococci recovered from the beach waters at Kuhio Beach and if swimmers at this beach were at an unacceptable risk of becoming infected with sewage borne pathogens. Results of a sanitary survey of the area did not indicate a likely sewage source but indicated that the most likely source of the enterococci bacteria was the Kapahulu storm drain water which is discharged from the end of the jetty which forms the eastern wall of Kuhio Beach. However, there was insufficient data to conclude that this storm drain system is the source of enterococci bacteria recovered from Kuhio Beach.

The primary goal of this study was to determine the concentrations of several types of fecal bacteria (fecal coliform, E. coli, enterococci, C. perfringens) in the Kapahulu Storm drain system and its impact on the quality of water in Kuhio Beach. Another major goal of this study was to simultaneously conduct a pilot epidemiological study to determine whether there was a measurable increase in the illness rate of swimmers at the beach as the concentrations of indicator bacteria in the water increased. Additional goals to this study included the determination of the sources of indicator bacteria in the storm drain and to analyze the sediment and water samples from the storm drain for toxicity as well as for the presence of specific toxic chemicals using a new enzyme-immunoassay test.

The Kapahulu Storm drain collects storm water from the hotel area of Waikiki as well as the urbanized area near Kapahulu Avenue and channels these sources of water under the jetty near Kuhio Beach where it is discharged into the ocean. Sampling sites were selected so storm water could be characterized as draining the Waikiki hotel area, the urbanized area, the mixing of these two sources before it is discharges as well as ocean water sites within and near Kuhio Beach.

Using a rapid method (Microtox) to detect for acute toxicity, storm water samples draining the urbanized sector of the Kapahulu Storm drain system were determined to be negative for toxicity whereas some storm water samples draining the hotel area were determined to be toxic. Toxicity detected in the storm water draining the hotel area was believed to be due to the higher use of products such as insecticides, fungicides, cleaners and solvents by the hotel industry. Moreover, hotels are more likely to discharge their waste and run-off water directly into the storm drain than are individual homes. An example of this is the milky white substance which had been periodically observed in the storm drain in the hotel area. We obtained a milky storm water sample which drained a hotel site, though we determined it was not toxic. The Department of Public Works, City and County of Honolulu, were informed and soon after found the source of the white substance. The department was able to determine that a hotel was illegally discharging its used water into the Kapahulu Storm drain system. This practice was put to a halt and the milky colored water has no longer been seen in the storm drain. Finally, toxicity was not detected in any of the ocean water samples indicating that the limited toxicity detected in the storm drain had been well diluted and was not causing a problem in the ocean.

At the end of this study, a new, simple to use, commercially available enzyme immuno-assay kit to detect for the presence of selected toxic chemicals became available. The major advantage of this methods is that no sophisticated equipment is required and non-chemists can run this test. This method was used to analyze water and sediment samples from some of the Kapahulu storm drain sites as well as from some streams for six toxic chemicals: 1) atrazine (herbicide), 2) benomyl/carbendazin (fungicide), 3) carbaryl (insecticide), 4) chlorpyrifros (insecticide), 5) 2,4 D (herbicide) and 6) PCP (wood preservative). The level of sensitivity varies for each chemical but is generally in the ppb range. Of the chemicals tested for, atrazine was the most frequently detected in the water samples from the storm drains and streams. Benomyl/carbendazin, carbaryl, chlorpyrifros, 2,4 D and PCP were also detected in the storm drain samples and at higher concentrations from the storm drain samples obtained from the hotel area than from the urbanized area. These results indicate that hotels use more of these products to control pests and vegetation than the Kapahulu urbanized area. It was concluded that this new method is feasible for screening water and soil samples for the presence of specific toxic chemicals.

The major focus of this study was to analyze water samples from several storm drain sites and several ocean sites, including Kuhio Beach for various types of fecal indicator bacteria. All storm drain sites were found to contain very high concentrations of the various indicator bacteria used in establishing recreational water quality standards. Of the fecal indicator bacteria tested, fecal coliform was detected at the highest concentrations with mean levels ranging from 851 to 24,081 CFU/100ml, followed by E. coli with geometric mean levels ranging from 572 to 9,291

CFU/100ml and lastly enterococci with geometric mean levels ranging from 241 to 3,975 CFU/100ml. Evidence was obtained to show that the wastewater from the Honolulu Zoo was not contributing to the bacterial load in the storm drain. Additional evidence was obtained to show that soil is a natural source of these fecal indicator bacteria and may be a major source of these bacteria in the storm drain,

The storm drain water was shown to be the major source of fecal bacteria recovered from the waters in Kuhio Beach. However, despite the high concentrations of the fecal indicators in the storm drain water, the storm drain water was effectively mixed with the ocean water at the discharge site at the end of the jetty at Kuhio Beach. Thus, the geometric mean concentrations of fecal coliform within Kuhio Beach was relatively low. For example, the concentrations of fecal coliform were well below the old water quality standard of 200 fecal coliform/100ml and the concentrations of enterococci were nearly always below the USEPA recommended standard of 35 enterococci/100ml. However, the geometric mean concentrations of enterococci often exceeded the stringent, current Hawaii standard of 7 enterococci/100ml. These same samples also contained very low geometric mean concentrations (0.2 to 0.5 CFU/100ml) of C. perfringens, supporting the data that the source of the indicator bacteria is not sewage.

Based on the USEPA study, of the source of enterococci is sewage, a geometric mean concentration of 7 enterococci/100ml should result in an incidence rate of approximately 10 diarrheal cases per 1000 swimmers. However, USEPA data also showed that if the source of enterococci is not from sewage, this relationship between concentrations of enterococci in recreational waters and predictable incidences of diarrheal diseases among swimmers is no longer reliable. In this regard, a pilot epidemiological study was conducted during the same period the quality of water at Kuhio Beach was being determined. The epidemiological study involved the completed questionnaires from 2,556 Kuhio Beach subjects. Analysis of the epidemiological data revealed no evidence of human health risk associated with recreational swimming at Kuhio Beach within the limits of the study. This means the actual level of illness due to swimming at Kuhio Beach was below the detectable limit of this pilot epidemiological study which was approximately 10 cases per 1000 swimmers. To determine the actual incidence rate, the epidemiological study would require a longer sampling period, with more subjects that could be followed over longer periods. The costs for these increased demands in the study design have been calculated. However, given the fact that the source of the enterococci recovered from Kuhio Beach is not from a sewage source but most likely from a storm drain and given the fact that Hawaii has environmental (soil) sources of enterococci, the USEPA study of predicting an increase of diarrheal diseases as the concentrations of enterococci in recreational waters increases is not applicable at Kuhio Beach.

TABLE OF CONTENTS

MICROBIOLOGICAL CHARACTERIZATION OF WATER AND SEDIMENT IN KAPAHULU STORM DRAIN SYSTEM AND AT KUHIO BEACH

A PILOT EPIDEMIOLOGICAL STUDY OF HEALTH RISKS ASSOCIATED WITH SWIMMING AT KUHIO BEACH

PROJECT SUMMARY REPORT

ASSESSING THE IMPACT OF THE KAPAHULU STORM DRAIN SYSTEM ON THE QUALITY OF WATER AT KUHIO BEACH AND THE HEALTH OF SWIMMERS USING THE BEACH

Roger S. Fujioka

Project Completion Report KSDS-1

March 1994

PREPARED FOR
State of Hawaii
Department of Health
Contract No.: ASO Log No. 92-613
Project Period: 1 April 1992-31 December 1993
Principal Investigator: Roger S. Fujioka

WATER RESOURCES RESEARCH CENTER
University of Hawaii at Manoa
Honolulu, Hawaii 96822

I. INTRODUCTION TO STUDY

This multi-phasic study is called the Kapahulu Storm Drain System (KSDS) Study because it focuses on determining the quality of the storm drain water collected by the Kapahulu Storm Drain System and the impact of this water on the quality of the water at Kuhio Beach as well as the health risk to swimmers at this beach. The project completion reports are comprised of five separate reports referred to as KSDS-1, KSDS-2, KSDS-3, KSDS-4, KSDS-5. The title and the objectives of each of these reports are summarized as follows:

1. KSDS-1. "Project Summary report: Assessing the impact of the Kapahulu Storm Drain System on the quality of water at Kuhio Beach and the health of swimmers using the beach"

The objectives of this report are (1) to take an overview of this entire study and (2) to summarize and integrate the results of the four separate, sub-projects which together comprise this study. This report will enable the reader to quickly determine the results of the entire study without having to read the details of each of the sub-project reports. This report is therefore called a "Project Summary Report" and will serve as an extended executive summary. However, for methods used, experimental design and results (data), the sub-project reports must be read.

2. KSDS-2. "Acute toxicity assessment of the Kapahulu Storm Drain System and its impact on the quality of water at Kuhio Beach"

The objectives of this sub-project are (1) to measure for acute toxicity in the water and sediment samples from the Kapahulu Storm Drain System and (2) to determine whether the discharge of this storm drain water near Kuhio Beach results in measurable toxic effects at Kuhio Beach. The rapid, microbiological test method (Microtox) was used to measure for acute toxicity. This method was selected because it is economical, fast, capable of analyzing many samples from different sites and also because it is one of the few methods which can be used to measure for acute toxicity in sediment (soil)-samples.

3. KSDS-3. "Evaluation of a commercially available enzyme-linked immunosorbent assay (ELISA) kit to monitor water and soil for toxic chemicals (pesticides, herbicides, fungicides and PCP)"

The objectives of this sub-project are (1) to evaluate the reliability and feasibility of using a commercially available enzyme-linked immunosorbent assay (ELISA) kit to screen water and soil samples for selected toxic chemicals and (2) to determine if toxic chemicals are detectable in streams and storm drains in Hawaii. The ELISA test kit produced by Ohmicron Company was selected because it uses magnetic particles as the solid phase to adsorb the specific antibodies to react with the toxic chemicals. This design allows for greater surface area and larger sample volume for the reaction of the antibodies and toxic chemicals to occur. This design allows for greater sensitivity and the application of this method to test soil samples for toxic chemicals.

4. KSDS-4. "Microbiological characterization of the water and sediment in Kapahulu Storm Drain System and at Kuhio Beach"

The objectives of this sub-project are (1) to determine the concentrations of various indicator bacteria (fecal coliform, E. coli, enterococci, C. perfringens and bacillus spores) in water and sediment samples which are being collected by the Kapahulu Storm Drain System, (2) (3) to determine the concentrations of these same indicator bacteria in ocean water sites near the discharge of this storm drain and within Kuhio Beach to determine the impact of this storm drain discharge on the bacterial quality of water at Kuhio Beach, (4) to determine the sources of these indicator bacteria recovered from the storm drain and (5) to provide the water quality data at Kuhio Beach in support of the epidemiological study. Fecal coliform, E. coli and enterococci bacteria were selected because these bacteria are used in the establishment of recreational water quality standards. C. perfringens was used because studies conducted in Hawaii have shown that this bacteria is a more reliable indicator of sewage contamination than the indicators used in the recreational water quality standards. Bacillus spores were used as an indicator of land (soil) run off.

5. KSDS-5. "A pilot epidemiological study of health risks associated with swimming at Kuhio Beach"

Well designed and definitive epidemiological studies require great investment in time, resources and costs. These conditions were not available for this sub-project as funds and time for completion of this study were limited. Thus, this study was started with full knowledge of this limitation and therefore the expected results were more exploratory rather than definitive. Thus, this was clearly a pilot epidemiological study with a primary objective to conduct a feasibility study, to specifically determine and measure critical parameters that would allow more refined estimates of the scope of work, and the amount of funds required to obtain health risk data considered definitive at any pre-selected level of certainty. Additional objectives of this sub-project were (1) to determine whether storm drain discharge poses a measurable health risk to users of Kuhio Beach, (2) to determine whether any detected health risks are associated with specific indicators of water quality and (3) to generate information useful to public officials and health planners concerned with health and sanitation.

II. PURPOSE OF THIS REPORT

The multiphasic nature of this study required different experimental designs and methodology. As a result, this study was divided into four different sub-projects. A decision was made to prepare a separate, detailed report for each of the sub-projects rather than attempt to consolidate each of the sub-projects into one comprehensive report. This has resulted in four separate reports, each detailing the methods and the results obtained. It is recognized that these individual reports may be too detailed for many readers and moreover, most readers want a single report to address all of the results of the study. This project summary report will serve that purpose. In addition, this report will provide an overview of water quality problems in Hawaii and in particular at Kuhio Beach.

III. WATER QUALITY AT KUHIO BEACH BASED ON OLD FECAL COLIFORM STANDARD

Kuhio Beach has for many years been a popular beach within the Waikiki Area. The popularity of this beach is due in part to the calm waters resulting from the man-made walls and jetties which ring this beach. Moreover, the frontage of this beach is sandy and is not blocked by a hotel as most of the other areas of Waikiki Beach. The quality of the water at this beach site has been monitored by the State Department of Health to ensure that this water meets established recreational water quality standards. From 1970 until 1988, the recreational water quality standard was based on a monthly geometric mean of 200 fecal coliform/100 ml. The quality of water at Kuhio Beach consistently met this standard and moreover, there was no clinical evidence that people swimming at this beach were reporting unusual incidences of diarrheal diseases normally associated with water which cannot meet recreational water quality standards. In summary, Kuhio Beach was not singled out as a beach which could not meet the recreational water quality standard based on the fecal coliform standard and as a result concerns at this beach were minimal.

It should be noted that the establishment of 200 fecal coliform per 100 ml as a water quality standard was not based on reliable epidemiological data and as a result, the number of fecal coliform in recreational water could not be used to predict the number of pathogens in the water and incidences of diarrheal diseases among swimmers. However, since the source of fecal coliform bacteria is feces of man and warm blooded animals, the presence of this group of bacteria in recreational water was used as an index of fecal contamination. Thus, as the numbers of fecal coliform in recreational waters increased, it was reasonable to conclude that the risks to swimmers would also increase. Therefore, fecal coliform concentrations in recreational waters were used as indicators of detectable risk, although the actual risk could not be predicted. Although the fecal coliform standard was considered to be superior to the total coliform standard it replaced, the use of the fecal coliform standard has been criticized even from the day it was implemented.

IV. USEPA'S NEW RECREATIONAL WATER QUALITY STANDARD

In response to the criticisms against the use of fecal coliforms as indicator of recreational water quality, the USEPA conducted a ten-year, microbiological and epidemiological study (Cabelli, 1983) which for the first time provided direct and valid evidence that concentrations of fecal coliform in recreational waters were unsatisfactory indicator of incidences of gastroenteritis diseases among swimmers using that water. However, this same study clearly showed that concentrations of Escherichia coli and enterococci bacteria were reliable indicators of risks associated with the use of recreational waters. As a result of this study, USEPA (1986) mandated that all U.S. states, and territories use E. coli and enterococci rather than fecal coliform to establish new recreational water quality standards. For marine recreational waters, the new USEPA standard was a monthly geometric mean of 35 enterococci/100 ml. For fresh recreational waters, the new standard was a monthly geometric mean of 33 enterococci/100 ml or 126 E. coli/100 ml.

The major advantage in the establishment of the new USEPA recreational water quality standard is that the standard is based on the results of an epidemiological study and the number of indicator bacteria in water can be used to predict number of swimmers who can be expected to become ill from diarrheal diseases. Thus, with this new standard, a measurable risk could be determined and health officials would then determine the acceptable level of risks for diseases and determine the water quality standard based on the acceptable risk.

In summary, with each concentration of indicator bacteria (eg. enterococci), there is a predictable number of swimmers who can be expected to become sick from diarrheal diseases. In 1984, USEPA initially recommended that the marine recreational standard be set at 3 enterococci/100 ml since this concentration of indicator bacteria predicted an illness of 6/1000 swimmers, the minimal acceptable level based on the assessment of most epidemiologists. However, the level of 3 enterococci/100 ml received such considerable opposition during the public hearing phase that USEPA in 1986 revised the standard to 35 enterococci/100 ml in marine waters which can be expected to result in an illness rate of 19/1000 swimmers. For epidemiologists, this is a very high level to establish as an acceptable risk. The state of Hawaii also concurred that a risk of 19/1000 people was too high to be acceptable and established as an acceptable level of risk as 10/1000 swimmers. Based on this reasoning, the state of Hawaii marine recreational standard was set at a monthly geometric mean of 7 enterococci/100 ml. Hawaii marine recreational standard is the most restrictive in all of the states in the US.

V. ARE THE NEW USEPA WATER QUALITY STANDARDS APPLICABLE TO HAWAII?

The new USEPA marine recreational water quality standards are based on data obtained from studies conducted on beach sites at New York, Boston Harbor and Lake Ponchatrain in Louisiana. All of these beach sites were susceptible to nearby discharges of sewage. Correlation of the number of indicator bacteria in the beach water and incidences of diseases among the swimmers were established at these three beaches. In establishing a new national water quality standard, several assumptions were made. If these assumptions are not valid, the application of results to another site is not valid. In this regard, there are several assumptions listed below which may not be applicable to Hawaii.

1. The assumption made is that environmental conditions between the USEPA test sites and Hawaii are similar enough that the USEPA results should be applicable to Hawaii.

Environmental conditions are known to affect environmental results. When environmental conditions at two sites are similar, the results obtained from one site can be reasonably applied to the second site. Conversely, when environmental conditions at the two sites are different, the results obtained at one site are less likely to be applicable to the second site. The USEPA test sites are beaches on the continental USA which are located in the temperate region of the world. Hawaii's beaches are part of an island located in the tropical region of the world. The difference in the environmental conditions between the USEPA test

sites and those in the state of Hawaii is greater than in any other coastal state. Thus, of all the 50 states, the assumption of applicability to all states is least likely for Hawaii.

2. With the use of fecal indicator bacteria to establish recreational water quality standards, it is assumed that the source of the indicator bacteria recovered from environmental waters is feces of man or warm blooded animals and there is no major environmental source of these indicator bacteria which is not directly related to feces.

In a series of studies conducted in Hawaii, Guam and Puerto Rico, the same indicator bacteria chosen for water quality standards are naturally present in the environment (soil, plant, stream waters). Since there is no evidence that these environments are contaminated with fecal matter, these sources of indicator have been concluded to be environmental in nature and reflect conditions in tropical island environments. Thus, in Hawaii, Guam and Puerto Rico, the assumption that there is no environmental source of the indicator bacteria cannot be made.

3. With the use of fecal indicator bacteria to establish recreational water quality standards, it is assumed that the indicator bacteria cannot multiply in the environment.

In studies conducted in Hawaii, high concentrations of the three indicator bacteria (fecal coliform, E. coli, enterococci) used to establish recreational water quality standards can be recovered at high concentrations in freshwater streams as well as the surface and subsurface of soil. Based on these results, Hardina and Fujioka (1990) have concluded that these indicator bacteria are multiplying in the soil environment.

In summary, three assumptions are made by USEPA to justify the application of the new USEPA standards to Hawaii. If any one of these assumptions cannot be made, the use of these same indicator bacteria to set water quality standards and risk assessments in Hawaii will be greatly weakened and may be invalid.

The documentation of environmental sources of fecal indicator in Hawaii by Fujioka has cast a doubt not only in the recreational water quality standards as written but also on the more basic issue of whether the use of these indicator bacteria is valid. Recognizing this dilemma, Fujioka has determined that Clostridium perfringens is superior to any of the USEPA recommended indicator bacteria to determine whether an environmental water is contaminated with sewage or feces. In light of this information, Fujioka has consistently communicated this dilemma to all of the regulatory agencies including USEPA and the State Department of Health. Appendix A is a copy of a letter written by Fujioka, as a member of the State Water Quality Advisory Committee to Mr. Brian Choy, chairman of that committee. The letter was written for the committee as a whole to comment on a letter of intent by Dr. Bruce Anderson that Hawaii should consider a more stringent standard than that proposed by USEPA. Fujioka's letter explains how conditions in Hawaii are different than in the continental USA and that the USEPA proposed standards are not directly applicable to Hawaii. That letter recommends against setting a stricter standard using the same indicator bacteria (enterococci) recommended by USEPA. This same reasoning was presented in a testimony by Fujioka (Appendix B) as a private citizen

during the public hearing held by the Department of Health before it implemented the new marine recreational water quality standard of a monthly mean of 7 enterococci/100 ml.

VI. WATER QUALITY AT KUHIO BEACH UNDER NEW USEPA STANDARD

Having accepted the new USEPA marine recreational standard of a monthly mean of 7 enterococci/100 ml, the state of Hawaii began to monitor the beaches of Hawaii under this new standard. Results of this monitoring program which began in 1988 showed that the quality of water at Kuhio Beach may not be able to meet the new state of Hawaii enterococci standard. WRRC was requested to determine the possible sources of enterococci recovered from the waters at Kuhio Beach. In response to this request from the Department of Health, WRRC conducted a short preliminary assessment of the quality of water at Kuhio Beach between November 11 - 22, 1991. The objective of this study was to assess the role of new beach sand as a source of indicator bacteria since new sand was just brought to Kuhio Beach and this seemed to correlate with increased levels of enterococci. The report of that study is included as Appendix C and concludes that both sand and the Kapahulu Storm drain should be considered as sources of elevated enterococci in the waters at Kuhio Beach. The low level of C. perfringens recovered from the Kuhio Beach water samples indicated that the source of the indicator bacteria was not likely to be sewage.

In summary, the quality of water at Kuhio Beach which consistently met the old fecal coliform standard could not consistently meet the new and restrictive standard of a monthly mean of 7 enterococci/100 ml. Does this mean that there has been an actual increase in the risk to swimmers at Kuhio Beach or does this change in water quality simply reflect a change in the water quality standard? There is no simple answer to this question since the fecal coliform standard has been shown by USEPA to be an invalid predictor of illness among swimmers. Thus, the old fecal standard was not a good way to determine health risks to swimmers. The new enterococci standard has been reported by USEPA to be a good predictor of illness among swimmers if the source of the enterococci is sewage. In a subsequent study (Calderon et al., 1991) the USEPA confirmed that when the source of enterococci in recreational water is from a non-point source such as storm drain which does not collect sewage, the levels of enterococci in the water is not a good predictor of illness among swimmers. Moreover, environmental sources of enterococci in Hawaii may invalidate the usefulness of this indicator bacteria to predict the illness rate among swimmers.

VII. MOTIVATION FOR PRESENT STUDY

Several questions were raised when the quality of water at Kuhio Beach could not be relied on to meet the new marine recreational water quality standard of a geometric mean of 7 enterococci/100ml. Data was not available to answer the following questions: 1) Is the source of the enterococci bacteria recovered from Kuhio Beach sewage, sewage contaminated storm drain or storm drain uncontaminated with sewage? 2) Is the wastewater from the Waikiki Zoo discharged into the storm drain? 3) Are there elevated levels of toxic chemicals in the Kapahulu Storm Drain system? and 4) Are the swimmers at Kuhio Beach at an unacceptable risk as a

result of the elevated concentrations of enterococci bacteria? To answer these questions the present study was initiated to determine the concentrations of several types of fecal bacterial indicators in the Kapahulu Storm Drain System and its impact on the quality of water at Kuhio Beach. Another major goal was to simultaneously conduct a pilot epidemiological study to determine whether there was a measurable increase in the illness rate of swimmers at Kuhio Beach as the concentrations of enterococci in the water increased. Additional goals of this study include determining the sources of indicator bacteria in the storm drain and analyzing water and sediment samples from the storm drain for toxicity and presence of selected toxic chemicals.

VIII. SUMMARY OF COMPLETED STUDY

As stated earlier, the study was comprised of several individual sub-projects and a detailed report was written for each of the sub-projects. The results of each of the sub-projects are summarized below.

A. Sampling Sites. Figure 1 shows the sites selected for this study. For a detailed description of these sites, see KSDS-2 and KSDS-4.

B. Assessment for Acute Toxicity (KSDS-2).

This sub-project monitored water and sediment samples from all storm drain sites and from ocean water samples obtained from Kuhio Beach for acute toxicity using the Microtox method. This method is rapid (15 to 30 minutes per sample), economical, reproducible and has been tested against more than 1,300 toxic chemicals. This method is primarily used to screen many samples for acute toxicity or substances in water which have an immediate and severe effect (death) on aquatic life forms. In environmental waters, chemicals in water must reach a critical concentration before acute toxicity can be measured. This method will therefore measure for abusive amounts of contamination. This method will not detect for chronic toxicity or chemicals at low levels of contamination which may affect the reproductive cycle of aquatic organisms. Chronic toxicity tests are necessarily slower and much more expensive. In monitoring environmental waters such as storm drain, the first concern is to measure as many sites as possible to ensure that abusive levels of contamination which can be measured by acute toxicity is not occurring. Identification and subsequent control of these abusive sources of contamination must be implemented first. Moreover, low acute toxicity is often associated with chronic toxicity. Thus, screening for acute toxicity may also determine where additional testing using chronic toxicity tests should be done.

In this study, acute toxicity was not detected in storm drain water samples obtained from Sites 10, 11, 12 which represents storm water draining from the urbanized/zoo area of the Kapahulu Storm Drain System. However, acute toxicity was detected in some water samples from Sites 8 and 9 which represents storm drain water draining from the hotel area of the Kapahulu Storm Drain System. The higher incidence of acute toxicity detected from Sites 8 and 9 was probably due to higher use of products such as insecticide, fungicide, cleaners, solvents, which can have a toxic effect. Moreover, since the storm drain in hotel areas are closer to the hotels, the concentrations of these chemicals in storm drains within the hotel area can be

expected to be higher. The milky white substance which had been occasionally reported in the storm drain and in waters near Kuhio Beach was detected during our sampling schedule. This sample was not toxic although the freshness of the sample we obtained from the storm drain could not be determined. However, we notified the City and County of Honolulu and a crew from that agency was able to trace the milky substance to a hotel source where a pump was illegally set up to discharge waste into the storm drain. The hotel was forced to close up this illegal connection and the milky substance in the storm drain was never detected again.

All of the ocean water samples from Sites 1 - 7 were negative for acute toxicity indicating that the discharge of storm drain water into the ocean was not resulting in measurable acute toxicity in ocean water samples. Dilution with ocean water is one of the factors controlling for acute toxicity. Another factor is the low volume and sporadic times when the storm drain actually discharges into the ocean. Finally, samples were taken randomly and therefore acute toxicity events may have been missed.

None of the sediment samples from the storm drain systems were positive for acute toxicity. Chemicals responsible for toxicity have often been reported to be concentrated in the bottom sediment. However, this kind of data have often been obtained by measuring water and sediments for specific chemicals using laboratory methods to extract and measure chemicals. However, bioassays or tests using live organisms are needed to measure for acute and chronic toxicity. Use of live animals limits the kinds of tests which can be done. For example, it is difficult to expose fish to sediment and to determine the effect of the toxic chemicals or the sediment on the health of fish. The Microtox assay relies on extracting soil and sediment with water and testing the extract for toxicity. Soil samples are not extracted with solvents as the solvents are toxic in most bioassays. However, it is reasoned that substances which will elute from the sediment and become soluble in the water will have the greatest impact on aquatic organisms. Chemicals which are tightly bound to sediments and do not become part of the water phase primarily affect only aquatic organisms which feed on the sediment.

In summary, the Microtox method has been determined to be a feasible test to be used to screen many samples for acute toxicity. One other advantage of this test is that all the reagents can be stored in the freezer until ready for use and therefore this test can respond to emergency situation where a test needs to be done immediately. For most other bioassays, this is not the case. The limitation of the Microtox test is its sensitivity to all chemicals and the fact that it measures only acute toxicity. Reports in the literature show that water samples should be tested by a battery of bioassays tests since no organism has sensitivity to all toxic chemicals. Use of a battery of tests will increase costs and complicate the testing procedure. However, the Microtox test is amenable as one of the tests to be used in a battery of tests.

C. Evaluating the feasibility of detecting chemicals by ELISA. KSDS-3.

As a supplement to this study, a commercially available enzyme-linked immunosorbent assay (ELISA) kit was evaluated for its feasibility as a method to monitor water and sediments for toxic chemicals. This method uses the principle of antigen (toxic chemicals) and antibodies (ELISA reagent) to detect for the presence of toxic chemicals using a color test. The ELISA

method has been used in clinical medicine for many years and is now being applied to detect for toxic chemicals. This test method was used to measure water and sediment samples from the storm drain and from streams for six toxic chemicals: 1) atrazine, a herbicide. 2) benomyl/carbendazim, a fungicide. 3) carbaryl, an insecticide. 4) chlorpyrifos, an insecticide. 5) 2,4, D, a herbicide and 6) PCP, a wood preservative. The level of sensitivity varies with each chemical but is generally in the ppb range.

The results of using this new method showed that atrazine was detected at higher level (5.8 ppb) in water from Site 8 and much lower levels in water from Site 11 (0.15 ppb). This chemical was also detected in the sediments of the storm drain. Atrazine was below detectable levels in water and soil samples from Manoa Stream, Palolo Stream and Waimanalo Stream. Similar results were obtained when the other chemicals were tested for. For example, storm drain samples from the hotel tributary (Site 8 and 9) were also positive for benomyl/carbendazin, carbaryl, chlorpyrifos, 2,4, D and PCP. Thus, these chemicals were more prevalent in storm water draining hotel area than from urbanized area and was least prevalent in streams. These results indicate that the source of these toxic chemicals are primarily from products used in industry (hotel) and household to control pests and vegetation as well as to preserve wood. Hotel areas probably use more of these products than households. Moreover, the storm drains in hotels areas are closer to the source than are storm drains for urbanized areas.

The feasibility of using this new method to monitor storm water and streams for toxic chemicals was determined to be good. The advantage of this method is the ease of this new assay as compared to the conventional method where skilled toxic chemists, utilizing expensive equipment is required. This method eliminates the need for a toxic chemist and the expensive equipment as all reagents and equipment can be handled by a trained laboratory personnel. A major benefit of this method is that it allows people who are interested in contamination with toxic chemicals but who are not chemists, to measure for toxic chemicals. Other advantages are the cost per test and the ability to analyze soil samples and to run this test at the field site. The results correlated with traditional chemical tests. The disadvantages of this method are (1) that the sensitivity for each chemical differs and it is difficult to interpret the test when results are less than the detectable limit for that test. (2) the method detects compounds with structures very close to the toxic chemical designated and this may include metabolites of the toxic chemical which may not have the same toxicity level. The value of the ELISA test is its use as a screening test and to determine which samples should be analyzed by the more traditional and expensive chemical tests. Thus, the ELISA test and the traditional chemical tests should be complementary tests.

D. Microbiological Assessment. KSDS-4.

All of the storm drain sites (Sites 8 - 12) and the ocean sites (Sites 1 - 7) were characterized for their content of microbial indicators. Geometric mean concentrations of the following three bacteria have been used to establish recreational water quality standards: 1) fecal coliform (200 CFU/100 ml), 2) E. coli (126 CFU/100 ml), 3) enterococci (USEPA: 35 CFU/100 ml; Hawaii: 7 CFU/100 ml). All storm drain sites contained concentrations of indicator bacteria far in excess of the number of these same bacteria used in establishing recreational water quality

standards (Table 1). Concentrations of fecal coliform were usually the greatest with geometric mean levels ranging from 851 to 24,081 CFU/100 ml, followed by E. coli with geometric mean levels ranging from 572 to 9,291 CFU/100 ml, and lastly by enterococci with geometric mean levels ranging from 241 to 3,975 CFU/100 ml. The source of these bacteria was determined to be soil as similarly high concentrations of these same bacteria were recovered from soil samples within the zoo, outside the zoo and in the backyard of a private citizen. Thus soil is a natural source of these indicator bacteria in Hawaii and when it rains, bacteria in the soil is washed into the streams and storm drains.

In contrast to the above indicator bacteria, the geometric mean concentrations of Clostridium perfringens in the same storm drain samples were lower with geometric mean concentrations ranging from 5 to 147 CFU/100 ml. Higher levels of this bacteria are recovered from storm drain samples as compared to stream samples. Pet feces is one source of C. perfringens in storm drains. The relatively low concentrations of C. perfringens to the other indicator bacteria (fecal coliform, E. coli, enterococci) indicates that the primary source of these indicator bacteria is not sewage or feces and most likely soil since soil contains low levels of C. perfringens. Zoo waste was considered a source of fecal indicator bacteria in the storm drain. However, using dyes, it was shown that animal wastes from the zoo are discharged into the sewage system and not into the storm drain system.

Despite the high concentrations of indicator bacteria in the storm drain, the concentrations of these same bacteria recovered from Site 5, where the storm drain discharges into the ocean, were generally very low. Salinity readings at Site 5 indicate that the storm drain water was very well mixed with ocean water. Of all the ocean sites, concentrations of indicator bacteria were highest at Site 5 showing that the discharge of storm drain into the ocean is impacting the quality of water near Kuhio Beach. However, this impact is minimized by the apparent low flow volume of the storm drain and high dilution capacity of the ocean. Thus the geometric mean concentrations of bacteria at Site 5 based on 18 samples (Table 2) were 4.5 enterococci/100 ml, 10.3 E. coli/100 ml, 16.9 fecal coliform/100 ml and only 0.5 C. perfringens/100 ml. Site 5 was sampled on 164 separate days over a 16 month period as part of the epidemiological study. Under those conditions (Table 3), the geometric mean concentrations were 7.1 enterococci/100 ml, 9.4 E. coli/100 ml, 14 fecal coliform/100 ml, and 0.68 C. perfringens/100 ml.

The two major swimming sites within Kuhio Beach were Sites 1 and 2. During the 18 sampling regime, the concentrations of bacteria at Site 1 and 2 were similar (Table 2). The geometric mean concentrations were 2.0 to 2.3 enterococci/100 ml, 4.9 to 6.5 E. coli/100 ml, 10.5 to 12 fecal coliform/100 ml, and 0.2 to 0.3 C. perfringens/100 ml. Over the 16 month period of sampling, the geometric mean concentrations of indicator bacteria at Site 2 as compared to Site 1 (Table 3) were 3.5 to 4.9 enterococci/100 ml, 4.5 to 6.6 E. coli/100 ml, 7.0 to 9.2 fecal coliform/100 ml and 0.4 to 0.5 C. perfringens/100 ml. Thus, the concentrations of all indicator bacteria were consistently lower at Site 2 as compared to Site 1. These observations were consistent with the conclusion that the source of indicator bacteria in Kuhio Beach is the storm drain water. However, the bacteria in the storm drain water is effectively diluted by ocean water and the recovered concentrations of indicator bacteria are relatively low and will easily meet the

Table 1. Bacterial Geometric Means for Kapahulu Storm Drain System Sites (n = 18)

Site	Enterococci	E. coli	Fecal coliform	C. perfringens
8	890	9291	24081	11
9	241	671	1492	4.7
10	1510	2089	2145	31
11	376	572	851	58
12	3975	6270	5961	147

Table 2. Bacterial Geometric Means for Kuhio Beach Sites for Samples Taken Concurrently with Storm Drain Samples (n = 18)

Site	Enterococci	E. coli	Fecal coliform	C. perfringens
1	2.0	4.9	12.0	0.3
2	2.3	6.5	10.5	0.2
5	4.5	10.3	16.9	0.5

Table 3. Bacterial Geometric Means for Kuhio Beach Sites for Samples Taken Concurrently with Epidemiological Study (n = 300 - 304)

Site	Enterococci	E. coli	Fecal coliform	C. perfringens
1	4.9	6.6	9.2	0.5
2	3.5	4.5	7.0	0.4
5	7.1	9.4	13.9	0.7

old 200 fecal coliform/100 ml standard, and nearly always the 35 enterococci/100 ml USEPA standard. However, meeting the Hawaii marine standard of 7 enterococci/100 ml is more difficult. The rainy months which presumably increases the volume and load of bacteria at the ocean resulted in concentrations of enterococci exceeding the 7 enterococci/100 ml standard.

Elevated concentrations of enterococci, E. coli and fecal coliform were highest at Sites 1 and 2 during the same months when these bacteria were highest at Site 5. Moreover, the relative concentrations of these bacteria at Site 5, 1 and 2 were similar with a gradient showing highest concentrations at Site 5 followed by Site 1 and Site 2. Based on these results, we conclude that the source of indicator bacteria is the storm drain and this source of water is transported to Site 1 and then to Site 2. During this transport, there is dilution and inactivation resulting in the observed gradient. The consistently low levels of C. perfringens at Sites 1 and 2 also indicate that the source of the bacteria is the storm drain rather than sewage. Other ocean sites outside of Kuhio Beach contained very low levels of all indicator bacteria.

E. The epidemiological assessment. KSDS-5.

A total of 3,721 subjects at or near Kuhio Beach were initially questioned. A follow up interview for illness, primarily intestinal disorders, was done in three days. A total 2,556 persons completed the interview after the three day period for 68.8% completion rate. The participants included 51.4% males and 48.6% female. Moreover, 52.2% were from Japan while 24.3% were residents of US other than Hawaii and 11.5% were residents of Hawaii and the remaining 12.1 % were from other countries.

The results of this study revealed no evidence of human health risk associated with recreational use of Kuhio Beach. This conclusion is based on the number of interviews completed in this study and the three day incubation period. This translates to a background rate of between 10 to 20 per 1000 persons per three days. Since a truly detectable level of disease could not be established, the contribution of the concentrations of the various indicator bacteria in the water could not be determined. The level of illness at Kuhio Beach appear to be less than the level reported by the USEPA study. That study formed the basis for the current recreational water quality standard. However, for that study, a source of sewage was always present. In a subsequent study conducted by USEPA at a recreational site where sewage was not present, the levels of bacteria were not correlated to illness and less people become ill with diarrheal disease. The situation at Kuhio Beach is similar to the second USEPA study since the source of indicator bacteria is storm drain water and not sewage. Moreover, the levels of indicator bacteria were very low at Kuhio Beach and therefore the detection rate for illness would be much more difficult. Finally, the microbiological studies based on concentrations of C. perfringens indicated that the water at Kuhio Beach is not contaminated with sewage. Together, these results support the conclusion of the epidemiological study that the risk to swimmers at Kuhio Beach is low.

Further analysis by the epidemiological study has determined the cost required to conduct another epidemiological study which can detect lower levels of illness rate and any level of illness rate that is desired.

IX. FINAL ASSESSMENT

In conclusion, storm drain water contains high levels of indicator bacteria and the primary source of this bacteria appears to be soil. There is a need to document that indicator bacteria actually multiples in the soil environment of Hawaii. Since, storm drains are not receiving sewage, the concentrations of indicator bacteria in the storm drains cannot be correlated to the same health risk as when a body of water is contaminated with sewage and the source of indicator bacteria in the water is from sewage. Thus, the fecal indicator bacteria system which is recommended by USEPA is less applicable to Hawaii's environmental conditions. The results of this study lend further support that concentrations of C. perfringens in water is a better indicator of sewage contamination and therefore health risks.

The actual level of illness due to swimming in Kuhio Beach was below the detectable limit of this pilot epidemiological study. However, if the rate of illness was unacceptably high,

the results of the epidemiological study would have detected it. This raises the questions as to what should be done to the storm drain since it is the source of the indicator bacteria in Kuhio Beach. Since the results of our epidemiological study could not detect a health risk among swimmers at Kuhio Beach, one reasonable conclusion is that the current conditions of allowing storm water to discharge into Kuhio Beach is satisfactory. However, prudence states that it is not a good decision to allow a storm drain to discharge its water so close to a swimming area. The answer to what should be done about the Kapahulu Storm Drain System may require more information. Is the result of more sensitive epidemiological study required so a level of disease risk can be established? Or will the answer require an engineering/ safety and economic solution. To improve the situation, the storm drain should not be discharged at the current site, if another site can be found which is feasible and will not result in worse health risks. Short of that, an assessment should be made to discharge the storm drain farther away from Kuhio Beach so less of this water contaminates the water at Kuhio Beach. This may involve changing the discharge site such as extending it or changing the walls and jetties which surround Kuhio Beach. Finally, the waters within Kuhio Beach should also have better circulation.

X. REFERENCES

APHA. 1989. Standard Methods for the Examination of Water and Wastewater, 17th ed. Washington, D.C.: American Public Health Association, Inc.

Bisson, J.W. and Cabelli, V. 1979. Membrane filtration enumeration method for Clostridium perfringens. *Appl. Environ. Microbiol.* 37:55-66.

Cabelli, V.J. 1977. Clostridium perfringens as a water quality indicator. Special Technical Publ. 635. A.S.M.T.

Cabelli, V.J. 1983. Health effects criteria for marine recreational waters. EPA-600/1-80-031.

Calderon, R.L., E.W. Mood, A.P. Dufour. 1991. Health effects of swimmers and nonpoint sources of contamination water. *Int J Env Health Res.* 1:21-31.

Cheung, W.H.S.; Chang, K.C.K.; Hung, R.P.S. 1990. Health effects of beach water pollution in Hong Kong. *Epidemiol Infect.* 105:139-162.

Dufour, A.P. 1984. Health effects criteria for fresh recreational waters. EPA-600/1-84-004.

Fujioka, R.S. 1983. Stream Water Quality Assessment Based on Fecal Coliform and Fecal Streptococcus Analysis, Hawaii: Water Resources Research Center Technical Memorandum, Report No. 70.

Fujioka, R. 1990. Evaluation of Clostridium perfringens as a suitable indicator for recreational water quality standard. Project completion report to Hawaii Department of Health.

Fujioka, R.S. and Shizumura, L.K. 1985. Clostridium perfringens, a reliable indicator of stream water quality. *J Water Poll Control Fed.* 57:986-992.

Hardina, C.M. and Fujioka, R.S. 1990. Soil: The environmental source of E. coli and enterococci in Hawaii's streams. Accepted for publication on "Environmental Toxicology and Water Quality."

Hawaii State Dept. of Health. 1990. Chapter 54. Water Quality Standards.

Hawaii State Department of Health. 1991. Identification of bacterial, sediment and debris loads in the Kapahulu District, Oahu, Hawaii.

Honolulu Star Bulletin. October 16, 1991. Officials say zoo top suspect in Kuhio Beach contamination. P. A-5.

New Jersey State Department of Health. 1988. A study of the relationship between illnesses and ocean beach water quality: Progress Report.

U.S.E.P.A. 1986. Bacteriological ambient water quality criteria. Federal Register Vol. 51, No. 45, Friday, March 7, 1986.

World Health Organization/United Nations Environment Program. 1977. Health criteria and epidemiological studies related to coastal water pollution. Copenhagen: WHO Regional Office for Europe.

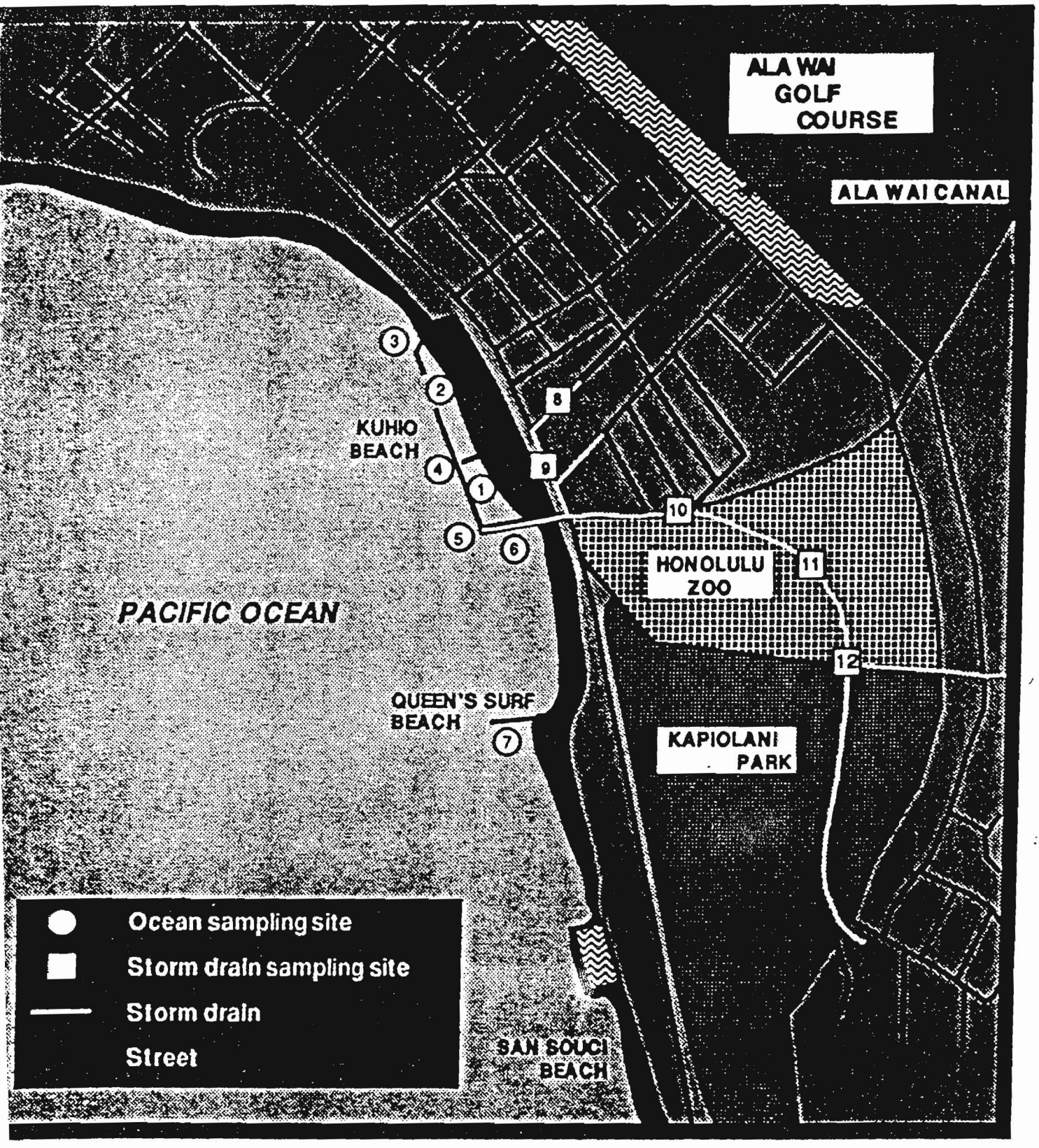


Figure 1. Sample Sites for Kapahulu Storm Drain System/Kuhio Beach Study

University of Hawaii at Manoa

Water Resources Research Center

MEMORANDUM
13 April 1987

TO: Mr. Brian Choy, Chairman
Water Quality Standard (WQS) Advisory Committee

FROM: Roger Fujioka, Member
WQS Advisory Committee

SUBJECT: Reply to March 17, 1987 letter from Dr. Bruce Anderson regarding health risks associated with swimming at Sandy Beach and measurements for enterococci bacteria in recreational waters and in zone of mixing for Hawaii Kai ocean sewage outfall.

As you know, I recommended and the WQS Advisory Committee accepted to accept EPA's recommended marine recreational water quality standard of 35 enterococci/100 ml (geometric mean for month). This recommendation by EPA is based on a 10 year study conducted by EPA at three beaches in New York City, Boston, and New Orleans (Lake Pontchatrain). Using superior epidemiological design and extensive microbiological analysis of water samples, this study for the first time showed a direct correlation of concentrations of enterococci bacteria in marine waters and swimming associated gastroenteritis diseases among swimmers. This correlation did not apply for concentrations of fecal coliform bacteria in marine waters, the current microbial indicator of water quality.

Based on the data published by EPA, Dr. Bruce Anderson, DOH Environmental Epidemiologist has made two recommendations to the Deputy Director for Environmental Programs. The two recommendations written in Dr. Anderson's letter of March 17, 1987 can be summarized as follows: 1. The acceptance of EPA recommendation that enterococci bacteria replace fecal coliform as water quality indicator for marine waters. However, Hawaii Department of Health should adopt a more stringent standard than the 35 enterococci/100 ml recommended by EPA. 2. Measurements for enterococci bacteria in zone of mixing for Hawaii Kai ocean outfall should be part of the NPDES permit.

I am in full agreement with Dr. Anderson's second recommendation that measurements for enterococci bacteria within the zone of mixing for ocean sewage outfall should be part of the NPDES permit. This request is in direct agreement with the WQS committee recommendation of adopting EPA's new marine recreational water quality standard based on concentrations of enterococci bacteria instead of fecal coliforms. Once this new marine recreational water quality standard is adopted, I assume that the PIE branch of DOH will include enterococci monitoring for any NPDES permit associated with discharge of sewage in marine waters.

I am in partial agreement with Dr. Anderson's first recommendation of accepting enterococci as the marine water quality indictor bacteria but requesting that the Department of Health accept a more stringent standard than the 35 enterococci/100 ml proposed by EPA. As was pointed out earlier, the WQS advisory committee has already recommended that the state DOH accept the EPA recommendation of setting 35 enterococci/100 ml as the new standard for marine recreational waters. The action of the WQS advisory committee is therefore in agreement with part of Dr. Anderson's own recommendation. However, Dr. Anderson's recommendation goes one step further and states that the DOH should consider setting a more stringent standard than that recommended by EPA because based on EPA's own data that marine recreational waters containing 35 enterococci/100 ml corresponds to a risk of 19 cases of gastroenteritis per 1,000 swimmers. I agree with Dr. Anderson's rationale that 19 cases of gastroenteritis per 1,000 swimmers is too high a risk factor for the DOH to accept. However, Dr. Anderson's rationale is based on the assumption that EPA's data is absolutely correct and moreover is directly transferable to Hawaii's environment. This was the same position that EPA originally took when EPA initially recommended that the marine recreational water quality standard should be 3 enterococci per 100 ml which corresponded to a more acceptable risk factor of 6 cases of gastroenteritis per 1,000 cases of swimmers. Thus, Dr. Anderson is arguing for the original proposal as stated by EPA, which we in Hawaii and most other states argued successfully against based on the reasoning that the data obtained by EPA was not directly transferable to all parts of the country.

All states are in the same dilemma, as pointed out by Dr. Anderson, of accepting the current EPA recommended marine recreational water quality standard of 35 enterococci/100 ml and in the process accepting a high risk factor of 19 gastroenteritis cases per 1,000 swimmers. However, this again accepts the assumption that EPA's data is directly transferable to all parts of the country, including Hawaii. I believe that EPA's data cannot be directly transferable to all parts of the US, especially Hawaii because the data was obtained from only three beaches (New York City, Boston, New Orleans). None of these beaches are similar to the conditions in Hawaii especially Lake Pontchatrain, the marine beach in New Orleans. I believe EPA's data that enterococci concentrations in water does correlate with incidences of gastroenteritis among swimmers. However, I believe that each region in the US has their own sources of enterococci bacteria which are entering the marine waters. Moreover, for many locations including Hawaii, these sources of enterococci bacteria are not from sewage. Thus, each state must determine their own background concentrations of enterococci in their water and must adjust the concentrations of enterococci in their water to set their own risk levels.

In actuallity, setting risk levels can only be done after an epidemiological study as conducted by EPA. If one takes the position that the EPA data cannot be directly transferable to Hawaii's condition, the only way to establish a true risk level for Hawaii is to repeat an epidemiological/microbiological study conducted in Hawaii. I cannot argue against this study since it really is needed. However, I would not be in favor of this type of study until it can be proven that the same study design as used by EPA be used. This is an expensive and labor intensive study. The investigators carrying out this study must be made well aware of the demands of this kind of study. It is not a kind of study that can be

simply contracted out. It will require, close and continuous attention to detail, thorough follow up and complete understanding of the many things that can go wrong.

In summary, it would be most useful to the WQS advisory committee and to the State of Hawaii if the appropriate office within the Hawaii State Department of Health would write an official position paper with regard to the new EPA proposed water quality standards. Some of the questions which this position paper should address would be: 1. The reliability of the study design, the methods used, the statistics used and the conclusions of the EPA study. 2. The acceptance that concentrations of enterococci bacteria but not fecal fecal coliform in recreational waters can be used to assess health risks associated with swimming. 3. Whether the same concentrations of enterococci with their corresponding disease risk as published by EPA can be directly applied to Hawaii beaches or whether some adjustment in these figures should be made. 4. A recommendation with regard to the complete, acceptance or the acceptance of a more stringent standard than that recommended by EPA for marine and fresh water quality standards in Hawaii. 5. Recommendation whether Hawaii should conduct its own epidemiological and microbiological study to determine the concentrations of enterococci in recreational water and risks to swimmers in Hawaii's beaches.

Public Testimony on Water Quality Standards
Roger Fujioka, Water Quality Microbiologist
Water Resources Research Center, UH
April 16, 1992

My name is Roger Fujioka. Professionally, I am a water quality microbiolgist with the Water Resources Research Center, Univeristy of Hawaii where I have been conducting research on water quality since 1972. Based on my 20 years of experience in Hawaii, I can say with confidence that I am very knowledgeable about the scientific and historical basis for the development of water quality standards. Moreover, I have accumulated data on water quality which directly relates to Hawaii's environment. I will be limiting my testimony to microbial water quality standards and briefly on using microorganisms to monitor for toxics in water, storm drains and sediments.

I wish to preface my testimony by complementing the Hawaii State Department of Health (DOH) for providing the revised documents for chapter 11-54 and 11-55 as well as the documents with the rationale for the proposed revisions. These documents show that the DOH has made great strides in implementing their program to interpreting their monitoring data and to make recommendations to improve on their monitoring program.

After reviewing the documents, I have come to the conclusion that many of the problems of water qualty identified by the DOH are symptoms of a more basic problem. Until the basic problem is addressed, DOH will be spending considerable time, energy and money without solving the problem. The symptoms identified by DOH are that several beaches can be expected to contain elevated concentrations of enterococci bacteria, exceeding Hawaii's recreational water quality standards. These beach sites can be characterized by poor circulation and being impacted by streams or storm drain flows which contain high concentrations of indicator bacteria (enterococci). The proposed solution is to post no swimming signs when the indicator bacteria counts become elevated.

My research results show that Hawaii's basic problem in assessing the quality of recreational waters is the presence of high concentrations of several indicator bacteria (fecal coliform, _E. coli_, enterococci) which are naturally present in the fresh water streams, storm drain flows, and soil of Hawaii. These are the same indicator bacteria used by USEPA to set recreational water quality standards with the assumption that these bacteria do not exist in the environment unless the environment has been directly contaminated with sewage or fecal matter. Based on studies I have conducted in Hawaii and Guam, this assumption cannot be made for Hawaii and the most likely explanation is that

these indicator bacteria are growing in Hawaii's warm, moist, soil environment. If this assumption cannot be made for Hawaii, Guam and probably other tropical islands, the USEPA water quality standards may not be applicable for environments such as Hawaii. The basic question is whether the indicator bacteria recovered from streams and soils in Hawaii are true indicator of fecal contamination. Recognition and resolution of this problem must be addressed. For example, when and how will Hawaii implement USEPA current fresh water recreational standard of 126E. coli or 33 enterococci/100 ml. If this is an unattainable goal for most of the streams in Hawaii, there should be a reasonable explanation, other than to conclude that all streams in Hawaii are contaminated with sewage.

The solution I see is for DOH to engage in direct communication with USEPA to discuss and to come to some understanding about a problem which appears to be characteristic of tropical island environments. I have been communicating with USEPA about this problem. However, I have come to the conclusion that USEPA responds to action agency such as DOH and considers communication with University more on an advisory level. In conclusion, an assessment of the interpretation of indicator bacteria naturally present in Hawaii streams by DOH and USEPA is needed and will be addressing the basic problem rather than addressing the symptoms of the problem.

With regard to chapter 11-55, storm drain flows in Hawaii also contain high concentrations of indicator bacteria. The sources of these bacteria could be environmental or feces of animals. However, both these possibilities should be recognized in decisions for pretreatment if required. Finally, as a laboratory analyst, I wish to state that methods which can be used immediately when a suspected toxic discharge in a storm drain is suspected should be approved in the monitoring of storm water discharges. Reliance only on standard methods which are time consuming and cannot respond rapidly to emergency situations will not address many anticipated problems. A microbial assay which can rapidly detect acute toxicity in storm drain water is available and its use should be encouraged.

I. PROJECT TITLE: Preliminary Assessment of Water Quality at Kuhio Beach

II. PRINCIPAL INVESTIGATOR: Roger Fujioka
Water Resources Research Center
University of Hawaii

III. PROJECT PERIOD: November 11 - 22, 1991

IV. THE PROBLEM. New sand was recently introduced to Kuhio Beach in October of 1991. Prior to the introduction of new sand (October 12, 1991), the Department of Health determined that water samples from all seven sampling stations at Kuhio Beach (see Figure 1) contained concentrations of enterococci below Hawaii's recreational standard of 7 enterococci/100 ml (Table 1). However, immediately after the introduction of the new sand, the Department of Health detected elevated concentrations of enterococci as well as fecal coliform in water samples collected at these same sites on October 18 and 24, 1991. What caused the elevated concentrations of indicator bacteria on October 18 and 24?

V. PROBABLE CAUSE FOR INCREASE IN INDICATOR BACTERIA. There are at least two sources for the observed increased levels of indicator bacteria at Kuhio Beach. The first obvious source is the run-off from the Kapahulu Storm Drain which discharges into Kuhio Beach near site 5 (Figure 1). However, it did not rain during this sampling period and therefore run-off was limited. Moreover, low concentrations of enterococci at site 5 suggested that the storm drain was not the source of the indicator bacteria. The second source for the indicator bacteria was the sand which was being added to Kuhio Beach. We (WRRC) previously determined that high concentrations of indicator bacteria are present in the sand at Hanauma Bay Beach Park.

VI. PROJECT OBJECTIVE. The WRRC agreed to analyze water and sand samples on two separate days to determine whether sand at Kuhio Beach is a major source of indicator bacteria.

VII. EXPERIMENTAL DESIGN AND METHODOLOGY. Water and sand samples were collected from Kuhio Beach on November 12, 1991 (9:00 -11:30 AM) and on November 14, 1991 (8:30 -10:30 AM). No rain occurred on both sampling days. Sampling stations for water and sand are outlined on Figure 2 and are similar to those established by the Department of Health. One additional water sampling site (site 8) was included to represent water from the western end of Kuhio Beach. Dry sand samples were obtained from sites 9, 10, 11, 12. Sand from sites 9 and 12 were selected as controls or sand which did not represent new sand. However, due to the mixing of sand, it was not clear whether this assumption was true. Water and sand samples were analyzed for the following indicator bacteria:

1. Enterococci: used for establishing water quality standard.

2. *E. coli*: used for establishing water quality standard in fresh water but not in marine water.

3. Clostridium perfringens. Alternative fecal indicator bacteria which we determined could be used as a marker for sewage.

4. Bacillus spp. Group of bacteria which could be used as a marker of soil and perhaps storm drain run-off.

All samples were also analyzed for turbidity and phosphates to characterize the various run-off.

VIII. RESULTS AND CONCLUSIONS. Results are summarized in Table 2.

1. Water samples from sites 2, 3, and 8 collected on November 12 and from sites 1, 2, 3, collected on November 14 exceeded the 7 enterococci/100 ml standard.

2. Elevated concentrations of E. coli in water samples correlated with the elevated concentrations of enterococci.

3. Elevated concentrations of Bacillus spores in water samples generally correlated with the concentrations of enterococci. The presence of Bacillus spores in marine waters indicate that the water sample is contaminated with soil. Soil may be present in storm drain run-off or sand.

4. C. perfringens concentrations in all water samples were low (0-2 CFU/100 ml) indicating that the source of indicator bacteria was not sewage.

5. The water samples collected at site 5 contained low concentrations of all indicator bacteria indicating that the run-off from Kapahulu Storm Drain was not a major source of indicator bacteria during the sampling period.

6. All indicator bacteria were recovered from sand samples. However, the concentrations of enterococci were highest in sand samples. Thus, ocean water washing over the sand, or people carrying sand into the ocean can be expected to add indicator bacteria, especially enterococci, to the water phase in Kuhio Beach.

7. Turbidity and phosphate measurements were generally low and did not indicate any unusual condition.

In summary, these results must be considered preliminary as they represent only two sampling days. Moreover, the run-off from the Kapahulu Storm Drain was not characterized as to its bacterial content. However, we have previously documented that other storm drain run-off contain very high concentrations of fecal coliform, fecal streptococci, enterococci and E. coli. Thus, under rainy conditions, the run-off from the Kapahulu Storm Drain can be expected to be a major source of indicator bacteria for Kuhio Beach.

Figure 1

DOH Sampling Site

LOCATION MAP

SCALE: 1" = 600'

PACIFIC OCEAN

State Water Quality Standard: "Enterococci content shall not exceed a geometric mean of seven(7) per one hundred milliliters in not less than five (5) samples spaced equally over a thirty (30) day period."

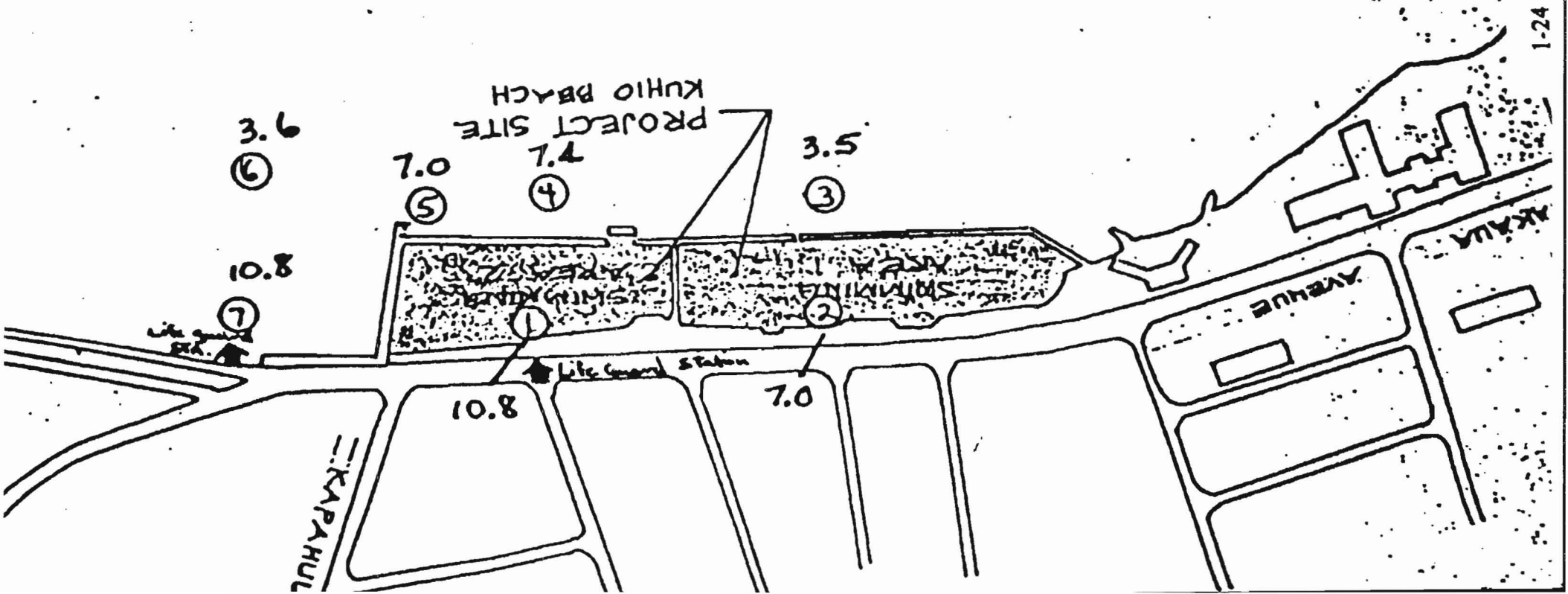


Table 1

DOH Data

MICROBIOLOGICAL RESULTS OF KUHIO BEACH WATER QUALITY MONITORING, OCTOBER 12-24, 1991

	STATION	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7
FECAL COLIFORM	OCT. 12	45.0	15.0	2.0	2.0	5.0	1.0	2.0
	OCT. 18	74.0	43.0	10.0	20.0	76.0	21.0	26.0
	OCT. 24	15.0	16.0	22.0	10.0	27.0	4.0	4.0
LOG AVERAGE		36.8	21.8	7.6	7.4	21.7	5.0	5.9
ENTEROCOCCUS	OCT. 12	2.1	1.0	0.5	2.0	6.4	2.1	2.1
	OCT. 18	44.0	17.0	3.8	20.0	8.6	44.0	44.0
	OCT. 24	13.7	20.0	22.0	10.0	6.3	0.5	13.7
LOG AVERAGE		10.8	7.0	3.5	7.4	7.0	3.6	10.8

Figure 2

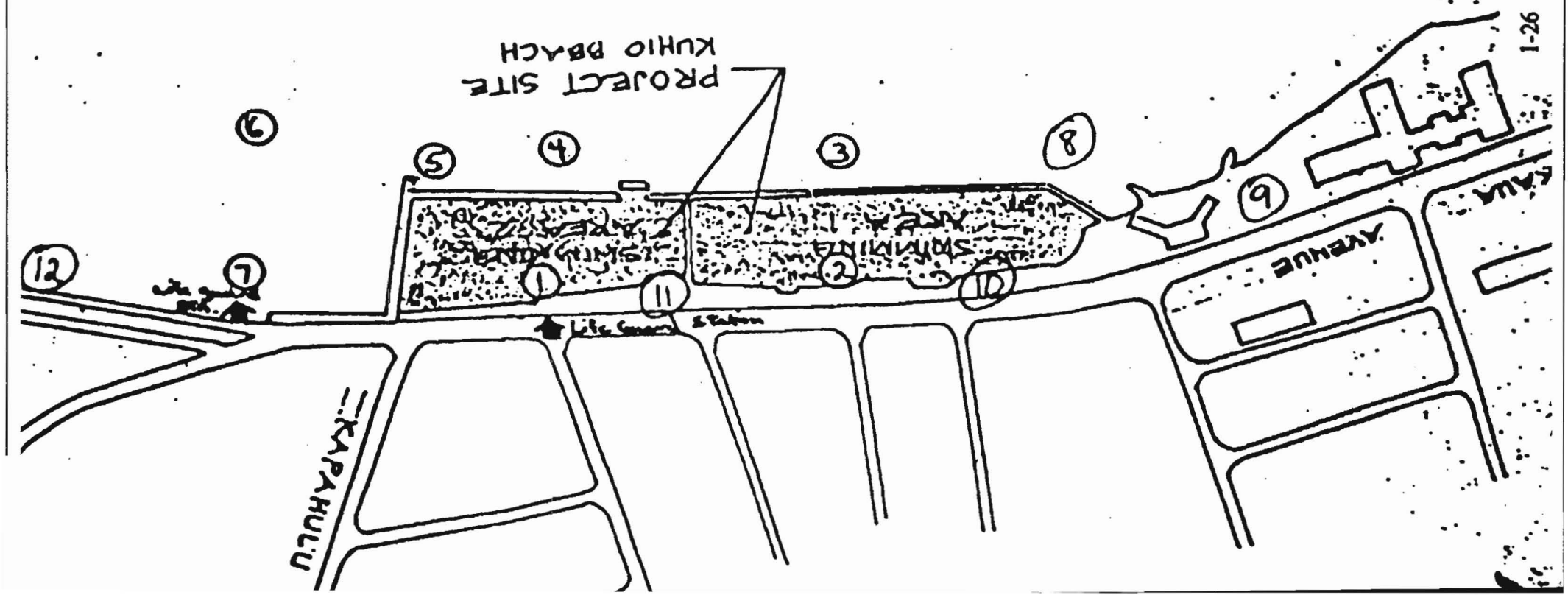
WRRC Sampling Site

LOCATION MAP

SCALE: 1" = 600'

PACIFIC OCEAN

State Water Quality Standard: "Enterococci content shall not exceed a geometric mean of seven(7) per one hundred milliliters in not less than five (5) samples spaced equally over a thirty (30) day period."



Kuhio Beach Results

11-12-91 CFU\100ml or g

Location	*E. coli*	*C. perfringens*	Enterococci	Bacillus spores	Turbidity (NTU)	Phosphorous (mg/l)
1	17	0	2	14	2	<.001
2	30	1	484	32	10	<.001
3	18	1	61	5	7	.001
4	6	1	1	0	1	<.001
5	0	0	2	3	2	<.001
6	4	1	8	7	1	.020
7	14	0	2	4	1.5	.002
8	2	2	5	14	2	.001
9 (sand)	1.6×10^5	80	2.48×10^4	104	nd	nd
10 (sand)	0	320	3.84×10^3	102	nd	nd
11 (sand)	304	80	160	19	nd	nd
12 (sand)	8	56	200	220	nd	nd

nd = not done

11-14-91 CFU\100ml or g

Location	*E. coli*	*C. perfringens*	Enterococci	Bacillus spores	Turbidity (NTU)	Phosphorous (mg/l)
1	14	0	10	1	2	<.001
2	34	0	13	4	3	.018
3	21	0	11	2	.9	<001
4	4	0	5	4	.5	.009
5	1	1	3	6	.3	<.001
6	6	0	1	1	1	<.001
7	7	0	5	1	1.5	<.001
8	1	1	1	1	2	<.001
9 (sand)	7.2×10^3	880	5.04×10^4	64	nd	nd
10 (sand)	0	0	8.8×10^3	128	nd	nd
11 (sand)	720	0	1.28×10^3	35	nd	nd
12 (sand)	8	24	184	50	nd	nd

nd = not done

ACUTE TOXICITY ASSESSMENT OF THE KAPAHULU STORM DRAIN SYSTEM AND ITS IMPACT ON THE QUALITY OF WATER AT KUHIO BEACH

Eric W. Faisst
Roger S. Fujioka

Project Completion Report KSDS-2

March 1994

PREPARED FOR
State of Hawaii
Department of Health
Contract No.: ASO Log No. 92-613
Project Period: 1 April 1992-31 December 1993
Principal Investigator: Roger S. Fujioka

WATER RESOURCES RESEARCH CENTER
University of Hawaii at Manoa
Honolulu, Hawaii 96822

I. MOTIVATION FOR STUDY

A. Point vs. Non-point Sources of Pollution

Point Source Pollution is defined by the Department of Water Works City and County of Honolulu and the Hawaii State Department of Health as "one in which pollutants enter a body of water from a specific, identifiable point, such as a pipe, ditch, tunnel, channel, or similar discrete conveyance."[1] For many years, the Hawaii State Department of Health has regulated and controlled point source discharges in compliance with the Federally-mandated National Pollutant Discharge Elimination System (NPDES) permit program.[1] Examples of point source discharges which require NPDES permits are sewage effluent and industrial discharges.

Non-point Source Pollution is defined as pollutants which come from many diffuse sources related to land activities (agriculture, urbanization, industrialization) and enter water from various unidentified points such as stormwater runoff and erosion of surrounding areas. Land surfaces, rooftops, parking lots and other paved surfaces represent initial collection areas for diffuse sources of pollutants. These pollutants are then drained into roadways, open drains and eventually to coastal waters by irrigation, rainfall or excess water used by man. With point source pollution under permits, non-point source pollution has recently been determined to be the largest contributor to water quality problems on Oahu. Hawaii's Non-point Source (NPS) Pollution Control Program utilizes best management practices (BMPs) and measures to reduce pollutant concentrations resulting from designated non-point sources.[1]

B. Stormwater as a Non-point Source of Pollution

Stormwater run-off has been identified as a major form of non-point source pollution and a major contributor to the degradation of many urban streams and rivers.[2-4] Heavy rainfalls wash soils from agricultural areas, and sediments, litter and trash from urban areas, and construction sites. The resulting runoff may contain sediments contaminated with pesticides, fertilizers, petroleum products, heavy metals, and other chemical contaminants. Additionally, street litter, trash, and agricultural residues contribute to the organic loading of stormwater runoff. These pollutants may contribute to the degradation of receiving waters and affect the aquatic biota within these waters. Public health concerns arise because coliform bacteria found in stormwater run-off indicate the presence of possible pathogenic bacteria.

C. Regulation of Stormwater Discharge

In November 1990 the U.S. EPA proclaimed new regulations that require a National Pollutant Discharge Elimination System (NPDES) permit for stormwater system discharges.[5] This program was first described in the 1972 Clean Water Act[6] and subsequently by amended sections of the Water Quality Act of 1987.[7] The regulations are a result of the information derived from the Nationwide Urban Runoff Program (NURP) study of about 100 stormwater outfall samples.[8] The studies demonstrated that non-point runoff from urban and other areas

contain elevated levels of physical, chemical, and biological pollutants. The implementation of stormwater regulations illustrates EPA's goal to effectively eliminate these sources of pollution.

The basic water quality criteria applicable to all waters in Hawaii state that "all waters be free of substances attributable to domestic, industrial, or other controllable sources of pollutants."[9] Oil and grease, suspended particulates and sediments, toxins, pathogens and other deleterious materials represent substances that, when in sufficient concentrations, may be harmful to plant and animal life. To ensure compliance with the basic water quality, all state waters are subjected to monitoring for acute and chronic toxicity. Acute toxicity tests monitor for concentrations of a toxic chemical or agent found in effluent or receiving waters that produce an immediate effect, such as death, on the test organism. A toxic substance that has a delayed long-term effect on the population of an aquatic organism is said to display chronic toxicity. To produce a toxic effect a chemical agent or its biotransformation product must reach the appropriate sites in the organism at a minimum concentration for a defined period. Chemical or physical properties of the agent, the exposure situation, and the organisms susceptibility determine whether or not a toxic event occurs.[10]

D. Approved method to assay for toxicity

Effluents, stormwater, receiving water and sediment take on toxic properties when concentrations of toxic agents (e.g., heavy metals, biocides, chemicals, particulates, biological products) reach a critical level. The presence of these toxic properties can reliably be determined only by measuring some deleterious effect on a living system such as animals, plants, or microorganisms. Tests using live systems are called bioassays. All live systems do not respond in the same manner to all toxic chemicals or toxic agents. Thus the selection of animals or live systems for a bioassay is important in reliably interpreting the results. Guidelines in selecting a live test system is to use a test system which is (1) sensitive to the toxic agents, (2) can be feasibly and reliably used in a laboratory assay, and (3) the results are acceptable for protecting the real living system (ecosystem, human population) of concern.

There are two classes of toxic bioassay tests. The first is called acute toxicity test and measures for the presence of toxic agents which have an immediate impact (usually death) on a population of a living system (animal). Since these effects are drastic, the test procedure is of short duration (hours to three days) and the toxic effect is usually death of the animal population added to the test water. The second is called chronic toxic bioassay test and measures for the presence of toxic agents which have a delayed or long term impact on a population of animals. These delayed impacts generally affect the subsequent generations of animals such as low reproducibility, smaller or weaker offspring.

Methods to be used to measure for presence of acute or chronic toxicants must be reliable, reproducible and comparable from location to location. Thus, for NPDES permits, toxicity tests must be approved by the EPA. Only a small number of tests utilizing live systems from higher forms of life (fish, insects, mollusca and invertebrates) have been approved by the EPA. The advantages of following the EPA guidelines are (1) standardized

tests will be used to determine acute and chronic toxicity, (2) the test species are from higher forms of life and therefore more applicable for predicting impacts on higher forms of life, and (3) the results of the tests can be compared on a national basis.

The disadvantages of strictly following the EPA guidelines are (1) some of the test animals selected are not available in all states, (2) the approved tests are costly, complicated and time consuming, (3) only a limited number of sites can be tested yearly, and (4) these tests require advanced preparation and therefore cannot respond to an emergency situation where an accident occurs and a sample is brought to the laboratory for immediate assay of toxicity.

Selection of a test species is often specific to the region. Species selected for bioassays should be adaptable to maintenance under laboratory conditions, should be readily available, and are indigenous to the waters under study. If such species are not available, circumstances necessitate the use of some other species, preferably one that is comparable to the indigenous specie. Additionally, comparative testing will be required to relate the sensitivity of the selected species with the indigenous species. Furthermore, the ecological relevancy of the alternative test organism to the actual environment must be considered.

Preparation of higher forms of life as test species (e.g. fish), requires considerable labor and monetary output. Fish selected for testing need to be acclimated to test conditions at least 10 and preferably 30 days prior to testing. Maintenance of the fish stocks is labor intensive. Storage tanks or ponds must be of sufficient size and well maintained. The water in which the fish are kept must be of sufficient quantity and quality such that they will remain in good condition. Care must be taken that the fish are not contaminated by harmful materials, and that the water is adequately aerated. The fish require food on a regular basis, as well as monitoring for any diseases or abnormalities.

E. Alternative Methods used for toxicity testing: The Microtox Test

Microorganisms or single celled organisms are the simplest system to use in a toxic bioassay test. In this regard, bacteria are widely employed to measure the effects of environmental contaminants.[11,12] The use of bacteria and other microorganisms as alternative assay organisms is based on the assumption that biochemical and physiological systems exhibit interactions between toxicants and biomolecules that are similar to all forms of life. However, due to individual differences in the structure and function of various life forms, the reactions to toxic chemicals, will vary from test animal to test animal. Bacteria, algae, and animals may therefore demonstrate similar reactions to some chemicals, but different reactions to other chemicals.[13] EPA has not approved the use of toxic bioassay using microorganisms for NPDES permits.

Microbial toxic bioassay tests offer several distinct advantages over the use of more traditional complex life forms as test species. Bacteria can be readily maintained and cultured in the laboratory. Their rapid growth and high densities allow toxic effects to be determined over a short time period in statistically significant numbers. Simple toxicity

bioassays that use microorganisms as the test organism provide a quick, inexpensive, and reliable method to collect toxicity data for toxicants contained in water and sediments.

Of all the microbial toxicity tests, the Microtox test developed by Microbics has been the most widely used and evaluated. The Microtox® bioassay[30] measures acute toxicity by recording decreases in light output of *Photobacterium phosphoreum*, a luminescent marine bacteria. Certain luminescent bacteria utilize a portion of their metabolic energy to convert chemical energy into visible light. When these luminescent bacteria are challenged by a toxic substance(s) their ability to produce light is reduced. A reduction in the bioluminescence indicates an alteration of cellular metabolism. The amount of light loss per sample is proportional to the toxicity of the sample concentration.[31] This test is simple, reproducible, rapid, precise, sensitive, cost effective, and results in a dose response relationship.[14,32,33]

Since its conception 15 years ago, Microtox® has become the most used test for assessing the toxicity of environmental and industrial wastes. The toxicity of over 1300 chemicals have been evaluated by the Microtox® test.[46] The assay has undergone extensive study including comparisons to acute bioassays with both fish and invertebrates for a large number of pure compounds and aqueous mixtures.[34-37] This extensive study of the Microtox® assay has demonstrated a general agreement between toxicity values determined by fathead minnow and *D. magna* acute assays and the Microtox® assay. However, Mazidji *et al*,[38] revealed that the Microtox® assay was less sensitive for secondary treated wastewater effluent samples than *Ceriodaphnia dubia*. Ankley *et al*,[39] likewise, demonstrated that Microtox" is less sensitive to the effects of ammonia in sediment pore water than *Ceriodaphnia dubia* and *Pimephales promelas*. Bennett and Cubbage[12], and Geisy and Hoke[13] found the *Photobacterium phosphoreum* bacteria, used in the Microtox® assay, to be a quick (~1 hr.), inexpensive, easy, and reliable test as compared to six species from higher life forms. Likewise, Pastorok and Becker[14] noted that the Microtox® assay demonstrated the greatest statistical precision for the least cost and the greatest sensitivity when compared to eight sediment bioassays comprised of diverse biological groups (e.g., amphipods, bivalves, and bacteria) covering different stages in the life cycle, and toxic effects or endpoints.

In summary the Microtox® assay demonstrates a relative sensitivity to many compounds similar to most species of fish, insects, crustaceans, protozoan and mollusk, and is recommended as the initial stage of a battery of assays scheme.[26,27]

A major disadvantage to the use of microorganisms is that the endpoint, or toxic effect, cannot be directly related to detrimental ecological effects. In a comparison of characteristics of sediment toxicity screening bioassays *Photobacterium phosphoreum* and *Spirillium* bacteria rated lower than the higher life form bioassays (e.g., *Pimephales promelas*, *Ceriodaphnia dubia*, amphipods, and mollusk) tested for ecological relevance, relatability to field effects, and relatability to regulatory standards.[13] Although bacteria represent an important niche in a marine ecosystem, it is uncertain whether the observed changes in metabolism measured by the microbial bioassay test would substantially and irreversibly impair the ecological function of higher forms of life in the ecosystem.[14]

F. Evaluation of Toxic Bioassays for Use in Hawaii

For many bioassays, the test organisms used are bred in laboratory conditions with little resemblance to the actual environment. Furthermore, even when standardized procedures are followed laboratory deviations are high, compared to analytical chemical determinations, since measurements are based on the response of biological rather than a physical, chemical, or biochemical, systems.[15,16]

Monitoring for toxicity using only one test species is a cause for concern. No single bioassay test is sufficient in detecting the range of major toxic effects.[17-19] The inability of a single bioassay to respond to the broad spectrum of toxicants has encouraged experts to develop a battery of bioassays scheme.[20-25] The battery of assays approach utilizes organisms representing several trophic levels to predict health and ecological effects of chemicals entering the environment. This method was adopted by the Washington Department of Ecology to evaluate Puget Sound sediments for environmental impacts[26] and by the National Water Research Institute in Canada to evaluate the quality of its lakes and streams.[27]

The EPA methods manual[28] provides a listing of species and bioassays that may be used for monitoring purposes. The State of Hawaii selected several species (*Ceriodaphnia dubia, Tilapia mossambica, Penaeus vannamei* or *Penaeus monodon* and 5 species of sea urchins) as test organisms to determine the presence of toxicants in state waters.[29] At least one test species is used for toxicity monitoring and the species are rotated on a monthly basis. *Ceriodaphnia dubia* may be used in freshwater only. *Tilapia* and *Penaeus vannamei* may be acclimated for use in fresh, brackish, or marine waters, whereas *Penaeus monodon* and the sea urchins are used for marine and brackish waters.

G. Toxicants in Hawaii's Stormwater

The potential impact of stormwater runoff on the quality of Hawaiian waters was demonstrated in the 1980 study conducted by the Hawaii Department of Health (DOH).[40] The study delineated varying levels of metal concentrations resulting from runoff from commercial and residential areas in the Manoa drainage basin and in sediment samples from Manoa's catchbasin and Ala Wai Canal.

In 1990 the Water Resources Research Center of the University of Hawaii developed its capability of analyzing water and sediments for toxic properties using the Microtox® method. Using this assay system, McParland[42] sampled fourteen water samples from various sites comprised of industrial, commercial and residential areas to determine the impact of storm drain effluents on receiving waters. The results of this study showed no acute toxicity in the storm water samples tested. In a study focused on the Ala Wai Canal, Lum,[41] in 1992, demonstrated some sites of toxicity primarily in sediment samples. These results suggest that stormwater in Hawaii carry toxic products sporadically and sediments are the most likely places when toxicants are deposited over time.

II. GOALS AND EXPERIMENTAL DESIGN

In April 1992 the Department of Health funded the University of Hawaii to study the impact of the Kapahulu Storm Drain System on the quality of coastal waters at Kuhio Beach, and the potential health effects to individuals who came in contact with these waters. The goal of this present study is to determine the toxic properties of the Kapahulu storm drain waters and its sediment using the Microtox assay system and to determine the impact of this storm drain system on the receiving water within and near Kuhio Beach.

Five storm drain sites and seven marine water sites were selected for analysis to determine toxicity, using the Microtox" assay, for water, elutriate, and sediment samples. Testing began June 1992 and continued until September 1993.

III. METHODOLOGY

A. Sampling Area and Sampling Sites

Twelve sites were selected for bacteriological and toxicity testing. Map 1 identifies the 12 sampling locations. The study area allows for the determination of stormwater runoff from three distinct areas; Sites 8 and 9 drain Waikiki, characterized as hotel/commercial and Site 12 drains the Diamond Head residential area before flowing through the Honolulu Zoo (sites 10 and 11). Resulting stormwater runoff from the 5 storm drain sites (sites 8-12) is ultimately discharged into the ocean (Site 5) through an opening at the end of a stone pier. The pier extends out approximately 75 meters from the beach into the ocean waters. Water depth, at the pier's end, fluctuates between 1 to 2.5 meters, depending on tidal activity. The area surrounding the point of discharge is a popular boogie boarding spot for the local populace and visiting tourists.

Sites 1-7 represent marine water sites , while sites 8 to 12 are part of the Kapahulu Storm Drain System. The twelve sites were further classified based on study design and site location and grouped into five sets (A, B, C, D, and E).

B. Set A Sampling Sites. Marine water sites 1 and 2 comprise Set A. These two sites represent the enclosed Kuhio Beach area which can be expected to be impacted by storm drain discharges.

1. Site 1, within Kuhio Beach, is an enclosed swimming area approximately 100 x 50 meters, containing a body of water approximately one meter deep. A stonewall acts as a breakwall against the waves to create a placid swimming area. Stormwater from the Kapahulu Storm Drain is discharged at the end of stone pier immediately outside the south eastern corner of the breakwall. A breach in the wall marks the only opening in the break wall surrounding Site 1, and this opening is adjacent to the stormwater emission site. The pier encloses the water in Site 1 on its eastern border.

2. Site 2 is a semi-enclosed, shallow bathing area to the west of Site 1. Site 2 is exposed to the open waters via an opening, approximately 25 meters in width, in the center of the breakwall. Similar to site 1, the waters within this enclosure are approximately 1 to 1.5 meters deep. Tidal action carries stormwater discharged from the pier at Site 5 to Site 1 and 2.

C. Set B Sampling Sites. Marine water sites 4, 5, and 6 comprise Set B. These three sites represent the ocean waters outside of the enclosed Kuhio Beach area which can be expected to be impacted by storm drain discharges.

1. Site 4 lays outside of the break wall of site 2. This site has limited swimming activity and is fully affected by the ocean activity. Site 4 may be affected by the stormwater discharged at the pier's end by the tidal action along the face of the break wall.

2. Site 5 is the mouth of the storm drain system where stormwater discharges into marine waters. A three meter opening spans the western end of the pier allowing for the discharged stormwater to mix with marine waters.

3. Site 6 lays immediately east of Site 5. The stone pier demarcates the western border of site 6. Site 6 is on the opposite side of the mouth of the stormwater discharge but in an open area subjected to constant tidal action.

D. Set C Sampling Sites. Sites 3 and 7 represent marine water control sites located outside of the Kuhio Beach area and not expected to be impacted by the stormwater discharge from Kapahulu Storm Drain System.

1. Site 3 lays west of site 2 and is exposed to full tidal activity. Hotel and commercial business establishments are situated immediately west of Site 3.

2. Site 7 is at Queen's Beach approximately 200 meters east of the discharge area (Site 5). A stone pier borders site 7 on its western edge. Site 7 maintains lower numbers of swimmers and bathers as compared to sites 1, 2, and 3. This is considered an open beach site.

E. Set D Sampling Sites. Storm drain sites 8 and 9 demarcate the stormwater runoff originating from the hotel and commercial establishments in Waikiki.

1. Site 8 represents the only fresh water site of the 12 sites sampled. Site 8 is a covered storm drain located midway along Ohua Street. Access to the water is through a manhole cover. A curb side opening allows runoff to enter site 8 directly. Hotels sit along both sides of Ohua Street. Except for three separate events, the salinity level recorded for Site 8 (Table 4) was 0 parts per thousand (ppt) . Water samples taken from this site appeared milky white in color on several occasions over the course of the study.

2. Site 9 is on Kalakaua Avenue adjacent to Waikiki Beach. Runoff issuing from site 8 discharges into site 9 at the Ohua Street and Kalakaua Avenue intersection. Discharge from Site 8 joins with runoff from the hotel and commercial area, to the west of Site 9, along Kalakaua Avenue. Site 9 is a covered storm drain juncture accessible from the street surface via a man hole. Samples collected from Site 9 were brackish with an average salinity level of 14 ppt ranging from 0 to 25 ppt (Table 4). The brackish characteristic of the water resulted from the close proximity of Site 9 to the ocean outlet and the subsequent salt water intrusion.

F. Set E Sampling Sites. Sites 10, 11, and 12 represent stormwater samples entering and emanating from the Honolulu Zoo storm drain system.

1. Site 10 is situated in the parking lot of the Honolulu Zoo adjacent to Kapahulu Avenue. Site 10 is covered and accessible via a manhole. Site 10 demonstrated brackish water characteristics with an average salinity level of 16 ranging from 4 to 27 ppt (Table 5). The brackish characteristic of the water appears to result from salt water intrusion from the coastal waters entering the mouth of the storm drain system (Site 5).

2. Site 11 is located within the Honolulu Zoo. It is accessible from the ground surface through a metal grate. Site 11 water samples were brackish with an average salinity level of 15 ppt ranging from 0 to 28 ppt (Table 5). The brackish characteristic of the water appears to result from ground water or salt water intrusion from the coastal waters entering the mouth of the storm drain system (Site 5).

3. Site 12 is located east of the Honolulu Zoo on Monsarrat Avenue. Site 12 is an open drainage ditch which receives run-off from the Kapiolani Park, Waikiki Shell Auditorium, and the Diamond Head residential area. Site 12 water samples were brackish with an average salinity level of 12 ppt ranging from 4 to 20 ppt (Table 5). The brackish characteristic of the water appears to result from brackish ground water and salt water intrusion from the coastal waters entering the mouth of the storm drain system (Site 5).

G. Collection, storage, and treatment of samples

1. Water Samples

Twenty water samples were collected from each of the 12 sites between June 1992 and September 1993. Glass sampling bottles (150 ml) were acid washed and rinsed thoroughly with deionized water prior to sample collection. During collection sample bottles were filled to the top and covered with parafilm® in accordance with Microtox® protocol.[31] Concurrently, water samples were collected in separate bottles and analyzed for microbiological indicators.

A sampling regimen was established and samples were collected uniformly (Sites 8, 3, 4, 2, 1, 9, 5, 6, 7, 12, 10, and 11) throughout the course of the study. Collected samples were placed in coolers, covered with ice, and transported to the laboratory within 2 hours. Samples were stored at 8°C and assayed, without pretreatment, within 24 hours.

2. Sediment Samples for Eluate (Elutriate) Analysis

Sediment samples were collected by scooping up the sediment with a clean, sterile metal cup and transferring the soil into acid washed glass bottles. The remainder of the bottle was topped off with storm drain water and covered with parafilm®. Collected samples were placed in coolers, covered with ice, and transported to the laboratory within 2 hours.

3. Sediment Samples for Direct Sediment Analysis

Sediment samples were collected using a clean, sterile metal cup, attached to a pole, and transferred into acid washed bottles. The bottles were topped off with storm drain water and covered with parafilm®. Collected samples were placed in coolers, covered with ice, and transported to the laboratory within 2 hours. Samples were stored at 8°C and assayed within 24 hours.

4. The Microtox® test for Acute Toxicity

The Microtox® Model 500 Toxicity analyzer measures for acute toxicants in water, sediment, and soil eluates by using strains of luminescent bacteria, *Photobacterium phosphoreum*. Lyophilized bacteria are rehydrated in reconstitution solution. Aliquots of 10 µl of the cell suspension are transferred to test vials containing osmotically adjusted diluent (20 parts per thousand [ppt]) and equilibrated to 15° C using a temperature controlled photometer. The cell suspensions are then challenged by a sample of unknown toxicity. Light readings are measured before the sample is added and 5 and 15 minutes after sample addition. A reduction in bioluminescence indicates an alteration of cellular metabolism. The amount of light loss per sample dilution is proportional to the toxicity of that sample concentration.[31] A dilution of the sample demonstrating greater than 50% light loss or EC50 is used as an end point for toxicity measurements.

Water and elutriate samples of unknown toxicity are analyzed by the Basic and/or 100% tests.

The Basic Test is the standard test procedure used in the Microtox® assay for determining the toxicity of a sample. The luminescent bacteria is added to a predetermined number of cuvettes containing 500 µL of osmotically adjusted diluent. A time zero I_0 reading of the light produced in

each cuvette is recorded. Five hundred microliters (500 µL) of sample, in varying concentrations, is added to the cuvettes containing the reagent for final sample concentrations usually between 6.1875 to 49.5%. After five and fifteen minutes of incubation, the light output from the cuvettes are recorded.

The 100% Test procedure enables one to test nearly 100% of the samples rather than 49.5% of the samples by the Basic Test Protocol. In this procedure, the water samples are added to cuvettes containing 500 µL of diluent. Luminescent bacteria is added directly to the cuvettes containing the sample/diluent mixture. After five and fifteen minute incubation readings are recorded. The 100% Test method is more sensitive to operator technique, i.e., pipetting, and results may not be quite as precise. Light output of the reagent changes with time even without a toxicant. In the Basic Test the effect of the light drift is compensated by a correction factor. The correction factor normalizes the change of each sample dilution by measuring the change in light output in one or more controls, containing only diluent and reagent, between time zero (I_0) and the five minute reading (I_5). A correction factor cannot be calculated for the 100% Test and its variants because it does not include I_0 reading. Light levels of a sample are compared directly to the light level of a control at time$_t$.[31] However, for samples of low toxicity, the 100% Test is preferred since the concentrations tested are higher than those used for the Basic Test.

5. Analysis of water

Water samples were subjected to the Basic and 100% test procedures for marine and estuarine samples detailed in the Microtox® Operation Manual.[31] Five and 15 minute light readings were recorded and entered into the computer for data reduction. The measured light level values are converted to "gamma" values. Gamma values are the ratio of light lost to the light remaining after the reagent is challenged by the sample. A gamma value of 1 (ratio 1:1) correlates to a 50% reduction in light output.

The data results are presented in a dose-response relationship to determine the effective concentration (ECXX) that causes a particular percent of light loss. For example, the EC50 is the effective concentration of a sample causing a 50% decrease in the reagent light output under defined test conditions; those being exposure time and temperature.[31] The EC50 values are calculated for the samples assayed and are presented in Tables 10 - Appendix A.

The fresh water sample (Site 8) required osmotic adjustment with solid sodium chloride to 20 ppt. Osmotically adjusted diluent (salinity = 20 ppt) provided by Microbics Corporation was used as the control when testing fresh water samples. Sites 9 through 12 were brackish (salinity >5 ppt) waters exhibiting a broad range of

salinity up to 28 ppt. Samples registering salinity levels between 5 and 20 ppt were adjusted to 20 ppt with solid sodium chloride. In addition, a control diluent was prepared for each sample using clean filtered sea water and tripled distilled water. The salinity level of the sea water control was lowered, by adding tripled distilled water, to equal the sample's initial salinity level. Solid sodium chloride was then added to the adjusted clean sea water to raise the salinity to 20 ppt.

For samples exhibiting salinity levels greater than 20 ppt, a clean sea water control was prepared for each sample by diluting the clean sea water with triple distilled water to reach the salinity level equal to the sample's salinity level. Sites 1 to 7 demonstrated normal sea water salinity levels (32-35 ppt) and required no control preparations. Clean filtered sea water was used directly, without dilution, as the controls and diluent for sites 1 to 7.

6. Analysis of Eluate (Elutriate) of Sediments

Fifteen grams of sample were transferred to sterile erlenmeyer flasks containing 30 milliliters of Microtox® diluent, sealed with parafilm, and placed on a Burrell Wrist Action Shaker (Model 75) and mixed for forty-eight hours. After the forty-eight hour period, samples were centrifuged (IEC HN-S Centrifuge) for 20 minutes at 2500 rpms. The supernatant or sediment eluate or elutriate was extracted from the centrifuge tubes and transferred to clean, acid washed glass flasks (50 mL). Salinity levels were determined for each eluate sample and adjusted to 20 ppt if necessary. Two milliliters of sample were diluted, according to Microtox® protocol, with diluent and assayed. A total of six sediment eluate samples were assayed between August 1992 and May 1993 for sites 8, 9, 10, 11, and 12. Soil eluate and sediment samples from sites 1 to 7 were not assayed for toxicity.

Sediment eluate samples were subjected to the Basic and 100% test procedures for sediment eluate samples detailed in the Microtox® Operation Manual.[31] Five and 15 minute light readings were recorded and entered into the computer for data reduction.

Sediment eluate samples were prepared according to Microtox® protocol. However, slight moderations were made to the diluent to sediment ratio as outlined in the Microtox® Manual. The ratio of diluent to sediment used was 2:1 instead of the 4:1 ratio outlined in the manual. It was believed that a detectable toxic event was more likely to occur by testing a more concentrated diluent/sediment mixture.

7. Analysis of Sediment Samples

Four sediment samples were collected and assayed between January 1993 and September 1993 for sites 8, 10, 11, and 12.

Sediment samples were assayed according to the Solid-Phase Test protocol detailed in the revised Microtox® Operation Manual.[43] The Solid-Phase test procedure measures the light output of the luminescent bacteria, then mixes them in a slurry of diluent and sediment sample so they are exposed to particle bound toxicants. The bacteria are then removed from the mixture by filtration and light output is measured.

Sediment samples were centrifuged (IEC HN-S Centrifuge) for 20 minutes at 2500 rpms. The supernatant was extracted and discarded. The remaining soil sample was mixed using a sterile metal spoon. A measured amount of sample (0.3 grams) was transferred into plastic tubes provided by the Microbics Corporation. Solid-Phase diluent was injected into 15 plastic tubes, including the sediment containing tube, and dilutions (2:1 ratio diluent to sample) were prepared according to protocol.

The dilution tubes were inoculated with luminescent bacteria and incubated at 15°C for 20 minutes. Filter columns were inserted prior to the end of the incubation period, yet did not come in contact with the soil/diluent mixture. After the incubation period, the filters were pushed downward into the soil/diluent mixture. The filtrate was transferred to corresponding cuvettes in the Microtox® incubation block. Light readings were taken after 5 minutes and the results entered into the computer for data reduction. The EC50 values were calculated for each sample and are presented in Table 10 - Appendix A.

8. Analysis of Salinity & pH

Salinity and pH values for each sample were determined within two hours after sample collection. Samples below the necessary salinity level of 20 ppt were osmotically adjustment with solid sodium chloride and brought up to a salinity level of 20 ppt. Salinity was measured using a Reichert-Jung refractometer (Cambridge Instruments Inc.).

Following sediment eluate preparation, pH values and salinity levels were determined prior to testing. Sample adjustments were made as necessary.

The pH level for each sample was determined using an Orion Research microprocessor pH/millivolt meter 811. The pH values ranged from 6.83 to 8.37 for marine water samples (sites 1 - 7), 7.20 to 7.99 for brackish waters (sites 9 -12), and 7.51 to 8.04 for the fresh water sample (Site 8) (Tables 1 to 5). The Microtox® Manual indicates that samples demonstrating pH values below 6.0 or above 8.0 require adjustment with either sodium hydroxide or hydrochloric acid. Samples were initially tested without pH adjustment, as suggested by Querishi *et al*,[44] and Evereklian and Bulich,[45] who indicated that adjustments to the pH of water and sediment samples may dramatically affect the nature of the toxicants contained in the sediment samples.

Samples that initially exceeded the recommended pH range for testing did not demonstrate toxicity at elevated pH values. The samples were not adjusted to the recommended pH range for further testing. In this instance, it appears that the luminescent bacteria were not effected by pH levels that exceeded their physiological pH. Therefore, the samples seem less likely to demonstrate toxicity when within the bacteria's physiological pH range.

9. Toxicity Ranking Scheme

To assist in the interpretation of data a toxicity ranking scheme, (0 to 4+ toxicity) , was developed to categorize the toxicity levels for the water, sediment elutriate, and sediment samples. The rankings are determined using EC50 values and/or Toxicity Unit ranges which are the end points used by the Microtox System. The following ranking scheme delineates the water/elutriate and sediment samples;

Water/Elutriate

EC50 Range (Water/Elutriate) Percent Concentration	Toxicity Unit (TU)	Ranking Scheme	Classification
0 - 25	>4.00	4+	Highly Toxic
26 - 50	2.00 - 3.99	3+	Moderately Toxic
51 - 75	1.33 - 1.99	2+	Toxic
76 - 95	1.05 - 1.32	1+	Minimally Toxic
96 - 98	1.02 - 1.04	+/-	Possibly Toxic
99 - 100*	1.00 - 1.01	0	Non-Toxic

* Equivalent to EC50 > 100% for test

Sediment

EC50 Range (Sediment) Percent Concentration	Toxicity Unit	Ranking Scheme	Classification
0 - 0.499	>200	4+	Highly Toxic
0.500 - 0.999	100 - 199	3+	Moderately Toxic
1.000 - 1.499	67 - 99	2+	Toxic
1.500 - 1.999	50 - 66	1+	Minimally Toxic
2.000 - 2.500	40 - 49	+/-	Possibly Toxic
>2.500	<40	0	Non-Toxic

The results of the Basic Test for the water and elutriate samples are based on a 6.1875 to 49.5% concentration range, therefore, EC50 values falling within the 51 to 100% range and below 6.1875% are determined by statistical extrapolation using the Microtox's® computer program. The water and elutriate sample results for the 100% protocol are based on a 12.375 to 99% concentration range. Likewise, EC50 values below this range are statistically extrapolated.

Toxicity classification is based on the premise that a toxicity reading equal to or below 50% concentration, for water and elutriate samples, represents a toxic sample. For sediment samples, classifying toxicity requires additional information about sediment composition. Sediments composed of large sized particles, such as sand, are considered toxic when the EC50 value is below a 1% sample concentration. For sediment samples with a high clay content an EC50 value less than a 0.5% concentration is considered toxic. The development of a hierarchy of ranges allows for sites to be prioritized based on toxicity level.

Toxicity Units (TU) may be used in lieu of EC50 values. The inverse relationship between EC50 values and toxicity level may cause confusion: the lower the EC50 value the greater the toxicity, whereas TUs are directly related to toxicity such that higher TUs indicate higher toxicity. Additionally, TUs simplify data comparisons: a 20 TU (EC50 = 5) sample is twice as toxic as a 10 TU (EC50 = 10) sample. Toxicity Units are calculated by dividing 100 by the EC50 value.

IV. RESULTS AND DISCUSSION

Twenty water samples were collected from sites 1, 2, 5, 7, 8 -12, and sixteen samples from sites 3, 4, and 6, between June 1992 and September 1993. The sites depicted in Map 2 have been color coded to correlate with the frequency of detecting toxicity.

A. Sets A, B, and C - Marine Water Samples

The results of analyzing marine water samples are summarized in Tables 1,2, 3 and show that no toxicity was detected in any of the marine water samples (Sites 1 to 7) assayed. It appears that the discharge of stormwater near Kuhio Beach did not result in measurable toxicity in coastal waters. It is important to remember, however, that the marine waters did not demonstrate toxicity at a level that was detectable by the *Photobacterium phosphoreum* bacteria used in this bioassay. The sensitivity of the luminescent marine bacteria, *Photobacterium phosphoreum*, varies with the type of substances present in the water.[46] Walker[47] found *Photobacterium phosphoreum* most sensitive to inorganics and organics, respectively. In contrast, *Photobacterium phosphoreum* may not be sensitive to extremely hydrophobic compounds,[48,49] especially if the toxicity of the hydrophobic compound is lessened by the presence of dissolved organic carbon (DOC) in the waters.[50]

B. Set D: Hotel/Commercial Storm Drain Sites (8 & 9)

The results of analyzing Set D samples are summarized in Table 4 and show that the water samples collected from the hotel/commercial area, designated as Set D (Sites 8 & 9), recorded toxic events. Site 8 exhibited the highest frequency of toxic samples with 5 of 20, or 25% of the water samples assayed demonstrating varying levels of acute toxicity (1+ to 4+ toxicity). Site 8's August 11, 1992 sample exhibited the most toxic reading (EC50=13.6525%; 4+ toxicity). Site 9 exhibited toxicity in 2 of 20, or 10% of the samples tested. Sites 8 and 9 were the only sites to record detectable toxicity levels of the twelve sites tested.

Site 8 demonstrated toxicity in 3 consecutive samples, 8/11/92, 8/18/92, and 8/26/92. The highest toxicity level (4+) recorded for these three samples occurred on August 8, 1992, with a EC50 value (15 minute reading) of 13.65% (Table 10 - Appendix A). Toxicity levels at this site gradually decreased over the next two weeks (Figure 1a - Appendix B). The 8/18/92 and 8/26/92 samples exhibited 2+ toxicity. The 7/22/92 sample demonstrated a 1+ toxicity after 15 minute exposure time period (EC50 = 93.5042%), but did not exhibit a toxic response at the 5 minute exposure time period.

Some toxic materials affect the luminescent bacteria differently. Phenol causes immediate light loss (within 5 minutes) followed by a period of slight recovery of light output. Other categories of toxicants, such as bivalent metals, require longer periods of exposure (15 minutes).[31] The data presented in Table 10 - Appendix A suggests that the toxicants may belong to the latter category. The 15 minute readings demonstrated higher toxic responses for all toxic samples. This could possibly explain the toxic response observed for the 15 minute reading and non-toxic response for the 5 minute reading for the Site 8 July 22,1992 sample.

The July 29, 1992 sample collected between the July 22 and August 11, 1992 did not demonstrate a toxic response. The toxicants recorded a week earlier are no longer present and may have degraded, volatilized off, diluted, or leached into the sediment creating a non-detectable level of toxicity in the water sample. A salinity reading of 4 ppt was observed for the 7/29/92 sample (Table 4 - Appendix A). The remainder of the Site 8 samples recorded salinity levels of 0 ppt. Hinwood and McCormick[51] noted that the salt concentration (1 to 7% NaCl) in the assay environment may affect the toxicity of certain metals. The 12/14/93 sample, which recorded a salinity level of 10 ppt, did not demonstrate a toxic response. Six of the seven samples demonstrating toxicity were fresh water samples (salinity = 0 ppt). The remaining toxic sample recorded a low salinity level of 2 ppt.

It appears that a toxic substance(s) was discharged into the storm drain system or leached from the sediment, near site 8 between 7/22/92 and 8/11/92. The toxic substance remained in the storm drain for the next two weeks. Gradually, by substance degradation, volatization, adsorption to sediment particles, or by the dilution effect of subsequent runoff, the toxicity levels decreased to non-detectable levels.

The August samples collected from site 8 were milky white in color and the water produced suds when shaken. Grab samples were collected from the Hawaiian Regent sump and Ohua St. C&L at site 8, on July 8, 1993 . The August samples exhibited the same milky white color and emitted a "chemical" smell, similar to previous samples. After correcting for color interference[16] however, the samples were deemed non-toxic. The presence of a milky substance in the drainage system has been noted in the past. The October 30, 1991 issue of the Honolulu Star-Bulletin carried an article about an odorous milky substance fouling the Kuhio Beach swimming area.[52] The milky white substance was observed in the drain system and in the area inside the breakwater (Site 1) by contractors working on the drain system.

An exploratory sample was taken on January 25, 1993 at a storm drain access located on Ohua Street, near the Kuhio Street intersection, approximately 25 meters "up-line" from site 8. No toxicity was detected at this site, or at Site 8 on this date.

At Site 9 only two of 20 water samples collected, 6/29/92 (3+ toxicity) and 7/22/92 (4+ toxicity), were toxic. Toxicity observed at Site 9 did not correspond to the same days that toxicity was recorded at Site 8.

C. Set E: Honolulu Zoo Storm Drain Sites (10, 11, and 12)

The results of analyzing storm drain water at sites 10, 11, and 12 are summarized in Table 5 and show toxicity was not detected in the stormwater run-off entering the Honolulu Zoo storm drain system from the Waikiki Shell and Diamond Head residential area (Site 12), in the stormwater collected within the zoo area (Site 11), and in the stormwater exiting the zoo (Site 10). It appears that the stormwater from urbanized and zoo areas are not toxic.

D. Analysis of Sediment Eluate (Elutriate) Samples

Elutriate was prepared and extracted from sediment samples collected from the hotel/commercial storm drains (Sites 8 & 9) and from the urbanized storm drains (Sites 10, 11, & 12). Elutriate testing was not conducted for the marine water samples (Sites 1-7). A total of six elutriate samples from sites 8, 10, 11, and 12, and 3 elutriate samples from Site 9 were prepared for analysis between August 1992 and May 1993. None of the elutriate samples tested exhibited toxicity. (Table 6).

E. Sediment Analysis

Four sediment samples were collected from the hotel/commercial storm drain (Site 8), and from the urbanized storm drains (sites 10, 11, and 12), between January 25 and September 8, 1993 . The results are summarized in Table 7 - Appendix A and Figures 2a to 2e - Appendix B. Most of the sediment samples assayed produced some toxicity.

The results obtained from the Microtox® assay facilitate site comparisons by developing a ranking scheme. Ranking provides insight into which sites are "hot spots" or are potentially more toxic, and guides concerned individuals to direct their remediation

efforts accordingly. The average toxicity levels, derived for each site using log transformations, were compared and ranked from high toxicity to low toxicity. Site 8 demonstrated the highest average toxicity level (EC50 = 0.1849%, 4+ toxicity) followed by sites 11 (0.1984%, 4+ toxicity), 12 (0.4414%, 4+ toxicity), and 10 (1.8159, 1+ toxicity) respectively. Map 3 illustrates the sediment sample toxicity ranking scheme. Site 11 demonstrates higher average toxicity levels than sites 10 and 12, which are located down stream and up stream of Site 11, respectively. It appears that toxicants passing through Site 12 flow to Site 11 and contribute to the sediment toxicity in Site 11. However, Site 10 demonstrates the lowest average toxicity levels of all the sediment samples. The toxic effects found in sites 11 and 12 apparently do not progress to Site 10, either due to blockages within the system, settling out, or by volatilizing or degradation prior to reaching Site 10. Site 10 displays higher salinity readings than sites 11 and 12, indicating the possibility of sea water intruding into the storm drain system up to this point. The intrusive sea water may create a dilution effect thereby reducing the toxic levels in Site 10.

V. SUMMARY AND CONCLUSIONS

The Microtox assay system of detecting acute toxicity using luminescent bacteria was used to screen water and sediments from the Kapahulu Storm Drain and within Kuhio Beach. Although this assay system cannot detect all forms of toxicants, this method has been extensively used and shown to be able to detect the presence of numerous (>1300) types of toxic chemicals.[46] Using this method, toxicity was not detected in water samples obtained from sites within the Kapahulu Storm Drain system characterized as receiving water from an urbanized area (Site 12) and flowing through the Honolulu Zoo area (sites 10 & 11). These results indicate that storm drain water from the urbanized area of the Kapahulu Storm Drain System does not contain sufficient concentrations of acutely toxic chemicals.

Using the same Microtox method, toxicity was detected in water samples draining the Waikiki area (sites 8 & 9) of the Kapahulu Storm Drain System. Storm drain water from these sites were characterized as receiving water from the hotel/commercial area. Water samples from Site 8 had low salinity indicating that the storm drain water was not being mixed with environmental waters (shallow groundwater, ocean water). The color of the water samples obtained from Site 8 was sometimes milky and susdsy, indicating the presence of some man-made product, most likely a cleaning solution. This milky substance was tested and could not be documented as being acutely toxic. However, its presence at Site 8 is indicative that high concentrations of man-made products are entering the storm drain system from this hotel/commercial area. This was supported by the observation that 5 of 20, or 25%, of the water samples obtained from this site were toxic. Water samples from Site 9, which is located downstream of Site 8, had moderate salinity indicating that the storm drain water at this site was being mixed with environmental waters (shallow groundwater, ocean water). Toxicity was detected in 2 of 20, or 10%, of the samples obtained from Site 9. The lower the frequency of detecting toxicity in water samples at Site 9 may reflect the dilution of the storm drain water and perhaps the degradation of the toxicant as it flows from Site 8 to Site 9.

These results indicate that man's activity greatly impacts on the quality of the water in the nearby storm drains. It can be expected that the more densely populated and heavily used areas characterized as hotel/commercial will have many more man-made products which will enter the storm drain system than from a corresponding urbanized area. Based on this reasonable assumption, toxic substances entering storm drain systems are more likely to occur in hotel, commercial, and industrialized areas as compared to urbanized areas. However, the sporadic presence of toxicants can be expected in storm drains under all conditions.

Sediments obtained from the Kapahulu Storm Drain System were then analyzed for toxicity using the Microtox method. Initially, sediment samples from sites 8, 9, 10, 11, and 12 were extracted with water and the aqueous extracts called eluates or elutriates were tested for toxicity by the Microtox method. All elutriate samples were negative for toxicity indicating that the toxicants which can be readily eluted from these sediment samples were not present in sufficient levels to give a toxic effect. One limitation of the elutriate method is that it favors the detection of water soluble toxicants adsorbed onto the soil. Some toxicants are soluble in non-aqueous or organic solvents and remain adsorbed onto the sediment. However, for practical purposes, toxicants which remain adsorbed onto the sediment usually do not cause an adverse impact to the aquatic organisms, unless the organisms ingest the sediment.

The pore water for sediment samples has been found to provide better results than the elutriate method.[53,54] Pore water is the supernatant obtained following the centrifugation of a sediment sample. Unlike the elutriate, where an osmotic solution is mixed with the sediment sample, the pore water is the liquid found naturally within the sediment aggregate. Elutriates may give different results than test pore waters. Hoke *et al*,[53] noted that elutriates were often more toxic than pore waters. However, elutriates may be useful in determining the potential toxicity of resuspended sediments in the water column.[55]

To address the potential problem of toxicants which remain adsorbed to sediments, an alternative method in which the entire sediment is used in the Microtox method, was utilized. This solid-phase method of testing sediments for toxicity was used to analyze sediment samples from sites 8, 10, 11, and 12. Using this method the resulting measurements indicated that most of the sediment samples were toxic. However, the interpretation of this test can be ambiguous since non-specific adsorption of the bacteria on the sediment will be read as toxic effects. Moreover, the composition of the sediment determines the amount of non-specific adsorption of the bacteria. Generally, the higher the clay content in the sediment, the greater the chance for false positive. Adjusting for some background adsorption, it was concluded that sediments from sites 8, 11, and 12, but not Site 10, contained toxic material.

The results obtained indicated that acute toxicity was detected in the storm drain water draining from the hotel/commercial sector of the Kapahulu Storm Drain System. Moreover, there was some evidence that toxicants which were tightly bound to the sediment were present in the sediments of the storm drain system. Since the Kapahulu Storm Drain discharges into the ocean near Kuhio Beach, toxicity within the storm drain must be entering

the ocean. To determine whether acute toxicity could be measured in the ocean water samples within and near Kuhio Beach, marine water samples from sites 1 to 7 were analyzed for acute toxicity using the Microtox method. None of these marine water samples were determined to be toxic indicating that toxicants present in the Kapahulu Storm Drain are being effectively diluted and or degraded once it enters the ocean water.

VI. FINAL ASSESSMENT AND RECOMMENDATIONS

The Microtox method to detect for acute toxicity was used to monitor for presence of acute toxicity in the water and sediments of the Kapahulu Storm Drain System. Based on the results of previous reports as well as the results of this study, we recommend that this method be approved for screening environmental samples for presence of acute toxicity for the following reasons.

1. The Microtox method to measure acute toxicity is rapid, reproducible, economical, and requires small volumes of water and/or sediment for testing.
2. The method is feasible for environmental monitoring, especially to respond to emergency situations because all the reagents are quality controlled, pre-tested, stable, easily stored, and can be used immediately when required.

3. This method has been evaluated and toxicity level tested against more different kinds of chemicals (>1300) than any other assay.

4. The primary reason EPA has not approved the Microtox method is the use of the simple bacterial assay system which many believe is not comparable to higher forms of life. However, in many comparative tests, the results of the Microtox method correlate well with results of acute toxicity tests utilizing complex life forms, especially the standard fish (fathead minnow) tests. Therefore, the results of this method is applicable for environmental and human health assessments.

5. This method is also amenable to test for the toxicants associated with sediments either by eluting the toxicants from the sediment or by exposing the entire sediment to the bacteria in the Microtox system. In contrast, it would be difficult to assay the toxic effects of sediments to complex life forms.

6. A modification of the Microtox test can now be used to measure for the mutagenic effect of chemicals, by modifying the same Microtox instrument. Mutagenic effects are different from toxicity effects. Moreover, determination of mutagenic effects using complex life forms are extremely difficult, time consuming and very expensive.

7. A drawback to the use of the Microtox method is that it measures for acute toxicity and not chronic toxicity. Determination of chronic toxicity is important as it may have a long term impact. However, for most environmental conditions today, neither acute toxicity or chronic toxicity has been measured. Moreover, the first concern on the pollution of the environment is to ensure that no acute toxicity is present since acute toxicity is

generally an abusive form of environmental pollution. The acceptance of the Microtox method is a means for quickly determining the presence or absence of acute toxicity in all of the environments of concern. This approach is logical as the results of this testing procedure will determine which environments have evidence of toxicity. The results can be used to determine which environments should be seriously tested for chronic toxicity since this method is very slow and expensive. Thus, the use of the Microtox method should be viewed as complementing the use of chronic toxicity assays rather than replacing the chronic assay test.

8. It is now widely accepted that no single test is capable of detecting the toxicity of all chemicals. As a result, it is widely recommended that a battery of tests, or at least 2-3 systems be used in monitoring for toxicity. If 2-3 systems are required to test environmental samples for toxicity, it would not be feasible to use several bioassays which use complex life forms because of the complexity in handling higher forms of life, the length of these tests and the resulting expense. On the other hand, the use of simple systems, such as the Microtox test, is suitable for analyzing same samples with multiple tests.

Bibliography

1. Water Quality Management Plan for the City and County of Honolulu. September, 1990. Prepared by the Department of Public Works City and County of Honolulu and the Hawaii State Department of Health. Sections11, 12 & 13.

2. Field, R. and R. Pitt, April 1990. Hazardous and toxic wastes associated with urban stormwater runoff. Presented at the Sixteenth Annual Hazardous Waste Researc Symposium; Remedial Action, Treatment, and Disposal of Hazardous Waste. U.S. EPA, Risk Reduction Engineering Laboratory, Office of Research and Development, Cincinnati, Ohio.

3. Young, D. R., T.K. Jan, R.W. Gossett, and G.P. Hershelman. 1980. Trace pollutants in surface runoff. Coastal Water Research Project 1979-1980 Biennial Report. Southern California Coastal Water Research Project. Long Beach, CA.

4. Dutka, B.J., K.K. Kwan, and S.S. Rao. 1989. An ecotoxicological and microbiological study of the Yamaska River. National Water Research Institute, NWRI Contribution # 89-147. Burlington, Ontario, Canada.

5. Fed. Reg. Friday November 16, 1990, pt 2 40CFR part 122, 123, & 124.

6. Clean Water Act 1972

7. Water Quality Act of 1987, Sections 401 and 503.

8. Environmental Protection Agency. December 1983. Results of the Nationwide Urban Runoff Program. Water Planning Division, PB 84-185552, Washington, D.C.

9. Hawaii Administrative Rules. Department of Health. Title 11 Chapter 54 Water Quality Standards. October 1992. Section 11-54-04, a - d.

10. Casarett and Doull's Toxicology: The Basic Science of Poisons. Fourth Edition. M.O. Amdur, J. Doull, and C.D. Klaassen, eds. Pergamon Press, New York. 1991.

11. Walker, J. D. 1988. Relative sensitivity of algae, bacteria, invertebrates, and fish to phenol: Analysis of 234 tests conducted for more than 149 species. *Toxicity Assessment.* 3: 415-447.

12. Bennet, J. and J. Cubbage. February 1992. Evaluation of bioassay organisms for freshwater sediment toxicity testing. Environmental Investigations and Laboratory Services, Washington State Department of Ecology.

13. Giesy, J.P., and R.A. Hoke. 1989. Freshwater sediment toxicity bioassessment: rationale for species selection and test design. *J. Great Lakes Res.* 15(4):539-569

14. Pastorok, R.A. and D.S. Becker. 1989. Comparison of bioassays for assessing sediment toxicity in Puget Sound. U.S. Environmental Protection Agency Region 10, Office of Puget Sound. EPA 910/9-89-004.

15. Greene, J.C., W.E. Miller, M.K. Debacon, M.A. Long, and C.L. Bartels. 1985. Comparison of three microbial assay procedures for measuring toxicity of chemical residues. *Arch. Environ Contam. Toxicol.* 14:659-667.

16. Dutka, B.J., and K.K. Kwan. 1981. Comparison of three microbial toxicity screening tests with the Microtox tes. Bull. Environm. Contam. Toxicol. 27:753-757

17. Cairns, J., Jr. 1984. Multi-species toxicity testing. *Environmental Toxicology and Chemistry*, 3: 1-3.

18. Samoiloff, M. R. 1988. Toxicity testing of sediments: problems trends and solutions. *Aquatic Toxicology and Water Quality Management*, J.O Nriaqu and J.S.S. Lakshinarayana, TP 180 A38 V22.

19. Devillers, J., R. Steiman, F. Seigle-Murandi, P. Prevot, C. Andre, and J.L. Benoit-Guyod. 1990. Combination of single species laboratory tests for assessment of ecotoxicity of p-Benzoquinone. *Toxicity Assessment.* 5: 405-416.

20. Sloof, W., 1985. The role of multispecies testing in aquatic toxicology. In J. Cairns Jr. (Ed.), *Multispecies Toxicity Testing.* Pergamon Press, New York: 45-60.

21. Baudo, R., J.P. Giesy, and H. Muntau. 1990. Sediments: Chemistry and Toxicity of In-Place Pollutants. Lewis Publishers Inc., Chelsea, Michigan.

22. Clark, S. M., C.W. Barrick, and M.R. Samioloff. 1990. A bioassessment battery for use in an industrial setting: a new management approach. In *Toxicity Assessment: An International Journal*, 5:153 - 166.

23. Lenz, P., R.Sussmuth, and E. Seibel. 1989. A test battery of bacterial toxicity assays and comparisons with LD_{50} values. *Toxicity Assessment.* 4: 43-52.

24. Ross, P.E. and M.S. Henebry. 1989. Use of four microbial tests to assess the ecotoxicological hazard of contaminated sediment. In *Toxicity Assessment*, 4:1-21.

25. Dutka, B.J. and K.K. Kwan. 1988. Battery of screening tests approach applied to sediment extracts. *Toxicity Assessment.* 3: 303-314.

26. Betts, B. 1991. Sediment management standards. Washington State Department of Ecology. Chap. 173-204 WAC.

27. Dutka, B.J. 1988. A proposed ranking scheme and battery of tests for evaluating hazards in Canadian waters and sediments. National Water Research Institute, Environment Canada, Contribution # 88-80, Burlington, Ontario, Canada.

28. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (EPA 600/4-90/027, September 1991)

29. Amendment and Compilation of Chapter 11-55. Hawaii Administrative Rules. Department of Health. October 1992. Appendix D 5a-c.

30. Bulich, A. A. and D.L. Isenberg. 1981. Use of luminescent bacterial system for the rapid assessment of aquatic toxicity. *ISA Transactions*, 20(1): 29-33.

31. Microbics Corporation. 1992 Microtox® Manual No. 55H550-1. Carlsbad, CA 92008-8883.

32. Ross, P. 1991. The role of the Microtox® system in aquatic toxicity testing. Illinois Natural History Survey Report. Champaign, Ill.

33. Isenberg, D.L. 1993. The Microtox® test, a developer's commentary. M. Richardson ed., Ecotoxicology Monitoring. VCH Publishers, New York, NY, pp 3-15.

34. Dutka, B.J. and K.K. Kwan. 1982. Application of four bacterial screening procedures to assess changes in the toxicity of chemicals in mixtures. *Environmental Pollution*, Series A, 29: 125-134.

35. Bulich, A. A., M.W. Greene, and D.L. Isenberg. 1981. Reliability of the bacterial luminescence assay for determination of toxicity of pure compounds and complex effluents. *Aquatic Toxicology and Hazard Assessment*, STP 737, TA401 A54.

36. Nacci,D., E. Jackim, and R. Walsh. 1986. Comparitive evaluation of three rapid marine toxicity tests: Sea Urchin embryo growth test, Sea Urchin sperm cell toxicity test and Microtox®. *Environmental Toxicology and Chemistry*, 5: 521-525.

37. De Zwart, D. and W. Sloof. 1983. The Microtox® as an alternative assay in the acute toxicity assessment of water pollutants. *Aquatic Toxicology*, 4: 129-138.

38. Mazidji, C.N., B. Koopman, G. Bitton, and G. Voiland. 1990. Use of Microtox® and *Ceriodaphnia* bioassays in waste water fractionation. *Toxicity Assessment*. 5: 265-277.

39. Ankley, G.T., A. Katko, and J. Arthur. 1990. Identification of ammonia as a major sediment toxicant in the lower Fox River and Green Bay, Wisconsin. *Environmental Toxicology and Chemistry*, 9: 312-322.

40. Hawaii Department of Health, Pollution Investigation and Enforcement Branch, Environmental Proteciton and Health Services Division. Stormwater Runoff and Urban Street Contaminants. June 1980.

41. Lum, K.R.L.H. 1992. Screening of Ala Wai Canal waters and sediments using the Microtox® toxicity analyser. Thesis submitted to Graduate Division of Civil Engineering, University of Hawaii-Manoa.

42. McParland, T.L. 1991. Water quality analysis of stormwater samples obtained from the Island of Oahu. Water Resource Research Center, University of Hawaii-Manoa.

43. Microbics Corporation. revised December 1992 Microtox® Manual, Carlsbad, CA 92008-8883.

44. Querishi, A. A., R.N. Coleman, and J.N. Paran. 1984. Evaluation and refinement of the Microtox test. D.Liu and B.J. Dutka (eds.), *Toxicity Screening Procedures Using Bacterial Systems*. Marcel Dekker, New York, pp. 1-22.

45. Evereklian, G. and A.A. Bulich. 1991. Test method for the bioassessment of sediment toxicity using the luminescent bacteria toxicity test. Submitted to ASTM Subcommittee. Microbics Corporation, Carlsbad, CA.

46. Kaiser, K.L.E. and V.J. Palabrica. 1991. *Photobacterium phosphoreum* Toxicity Data Index, Water Poll. Res. J. Canada 26:361-431.

47. Walker, J.D. 1988. Effects of chemicals on microorganisms. *Journal WPCF*. 60(6): 1106-1120.

48. Hermans, J., F. Busser, P. Leevwangh, and A. Musch. 1985. Quantitative structure-activity relationships and mixture toxicity of organic chemicals in Photobacterium phosphoreum: the Microtox® test. *Ecotoxicol. Environ. Safety*. 9: 17-25.

49. True, C.J. and A.A. Heyward. 1990. Relationships between Microtox® test results extraction methods, and physical and chemical compositions of marine sediment samples. In *Toxicity Assessment*, 5: 29 - 45.

50. Cunningham, V.L., M.S. Morgan, and R.E. Hamrah. c. 1984. Effect of natural dissolved organic carbon on the toxicity of chemicals to aquatic microorganisms. Smith, Kline & French Laboratories. Swedeland PA.

51. Wagner, P. Milky storm-drain ooze fouls Kuhio Beach swimming area. **Honolulu Star-Bulletin**, Wednesday, October 30, 1991

52. Hoke, R.A., J.P. Geisy, G.T. Ankley, and J.L. Newsted. 1989. Sediment toxicity assessment in Maumee River and Western Lake Erie. *J. Great Lakes Res.*

53. Swartz, R.C., G.R. Ditsworth, D.W. Schults, and J.O. Lamberson. 1986. Sediment toxicity to a marine infaunal amphipod: Cadmium and its interactions with sewage sludge. Marine Environ. Res. 18(2): 133-153.

54. Chapman, P.M., R.N. Dexter, and E.R. Long. 1987. Synoptic measures of sediment contamination toxicity and infaunal community composition (the sediment quality triad) on San Francisco Bay. *Mar. Ecol. Prog. Ser.* 37: 75-96.

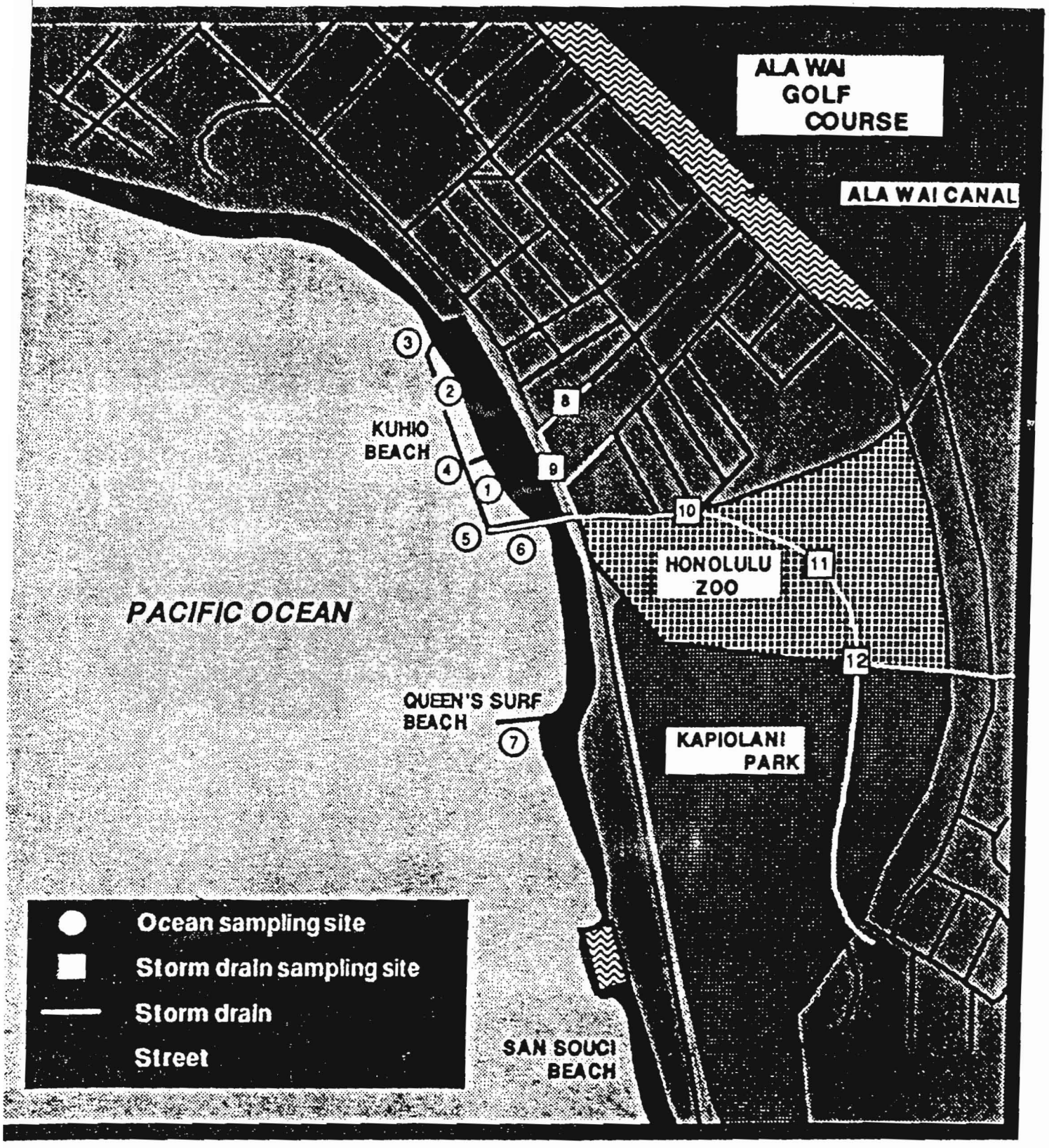


Figure 1. Sample Sites for Kapahulu Storm Drain System/Kuhio Beach Study

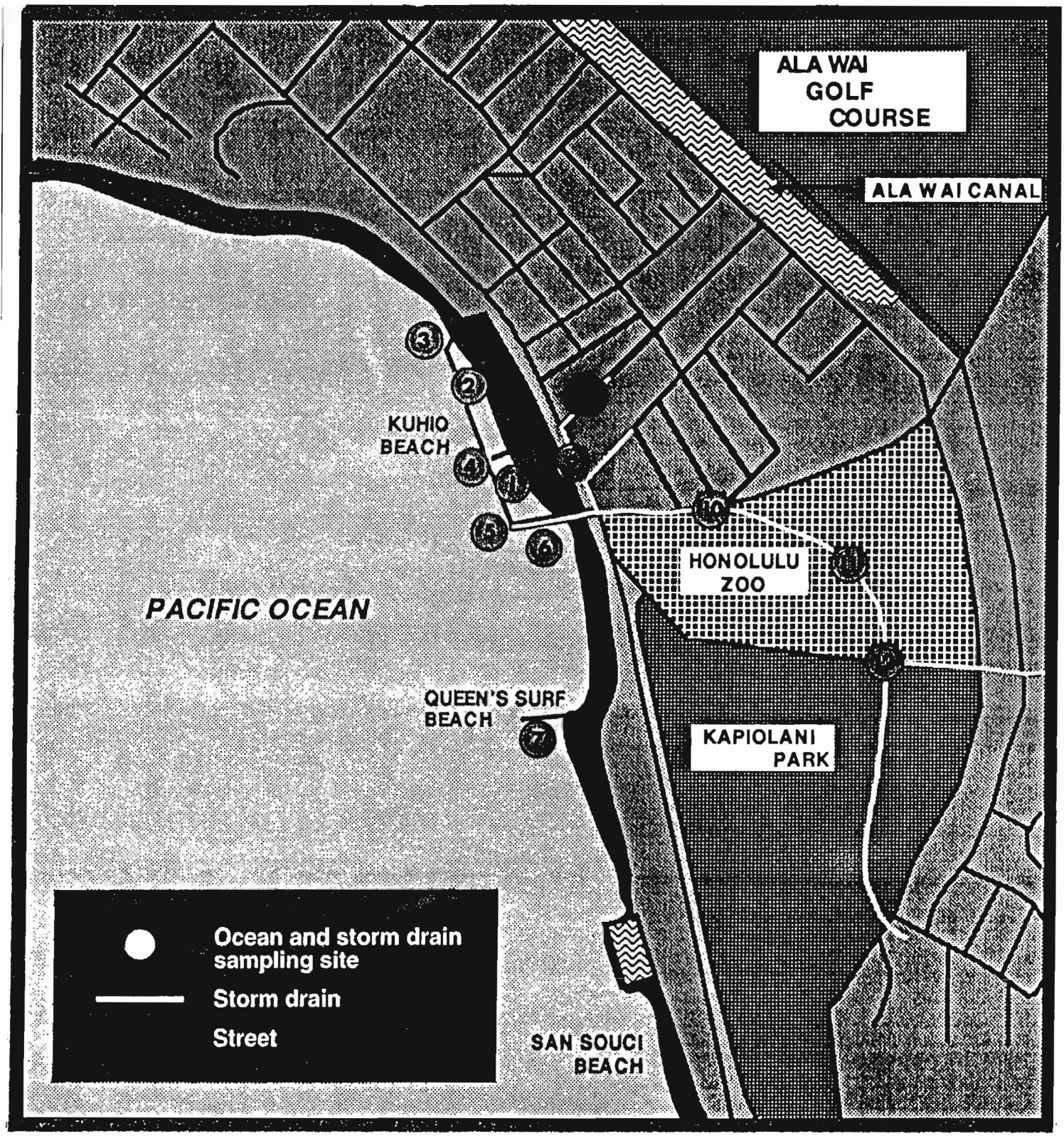


Fig. 2. Toxicity frequency in water at sampling sites.

KEY

- No Toxic Events
- Two (2) Toxic Events
- Five (5) Toxic Events

No Toxic Events – all except 8 and 9
Two Toxic Events – 9
Five Toxic Events – 8 (located above 9)

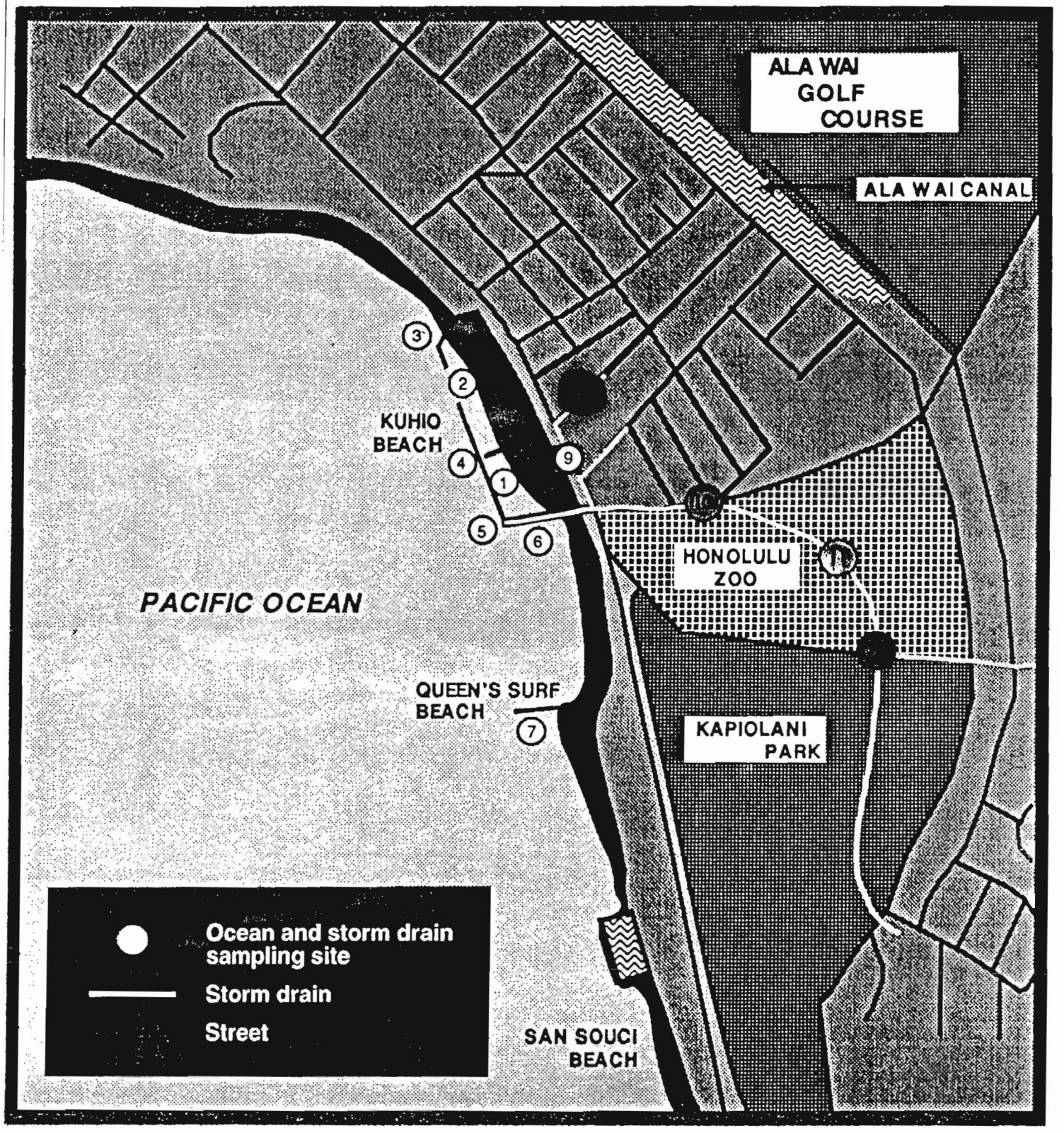
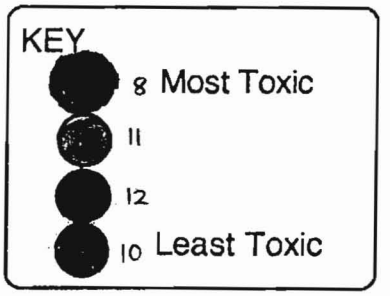


Fig. 3. Toxicity characteristics of sediment at sampling sites.



LEGEND
(Tables 1 - 10)

a = Presumably (0), test conditions not suitable
b = Light output increased (negative slope)
c = EC50 value not within the percent sample concentration range tested
d = Site 8.5, located north of Site 8 on Ohua St.
***** = 100% Test Protocol applied
♯ = Basic Test Protocol applied
@ = 30 minute reading recorded

Site 1 water sample results

Date	Salinity n = 14	pH n = 14	Toxicity 5 min	Toxicity 15 min
6/8/92	34	ND	0	0
6/17/92	ND	ND	0	0
6/29/92	30	ND	0	0
7/8/92	ND	ND	0	0
7/15/92	34	8.26	a	ND
7/22/92	34	8.02	0	a
7/29/92	34	8.18	0	0
8/11/92	34	ND	0	0
8/18/92	ND	8.11	0	0
8/26/92	ND	8.16	0	0
9/2/92	32	8.13	0	0
9/12/92	ND	8.07	0	0
10/7/92	34	8.01	0	0
10/26/92	33	7.26	0	0
11/10/92	34	8.06	0	0
12/14/92	34	7.99	0	b
1/25/93	34	8.24	ND	ND
3/22/93	34	8.21	ND	ND
5/17/93	34	8.09	b	0
9/8/93	ND	ND	ND	ND
Mean Value	34	8.06		

Site 2 water sample results

Date	Salinity n = 17	pH n = 14	Toxicity 5 min	Toxicity 15 min
6/8/92	34	ND	0	0
6/17/92	ND	ND	0	0
6/29/92	32	ND	0	a
7/8/92	32	ND	0	0
7/15/92	34	8.24	0	0
7/22/92	34	8.12	0	0
7/29/92	34	8.2	0	0
8/11/92	34	ND	0	0
8/18/92	33	7.86	0	0
8/26/92	34	8.2	0	0
9/2/92	34	8.09	0	0
9/12/92	ND	8.14	0	0
10/7/92	32	7.82	0	0
10/26/92	34	8.83	0	0
11/10/92	34	8.11	0	0
12/14/92	34	8.02	0	0
1/25/93	33	8.17	ND	ND
3/22/93	34	8.2	ND	ND
5/17/93	34	8.08	0	0
9/8/93	ND	ND	ND	ND
Mean Value	34	8.01		

SET A

Table 2. Salinity, pH, and toxicity results for Rondo Beach Sites 4, 5, 6

	Site 4 water sample results				Site 5 water sample results				Site 6 water sample results			
	Salinity	pH	Toxicity		Salinity	pH	Toxicity		Salinity	pH	Toxicity	
Date	n = 12	n = 11	5 min	15 min	n = 16	n = 14	5 min	15 min	n = 12	n = 12	5 min	15 min
6/8/92	34	ND	0	0	34	ND	0	0	12	ND	a	0
6/17/92	ND	ND	0	0	ND	ND	0	0	24	ND	ND	ND
6/29/92	34	ND	0	0	34	ND	0	0	24	ND	0	0
7/8/92	ND	ND	0	0	32	ND	0	0	6	ND	0	0
7/15/92	33	8.09	a	0	32	8.01	0	0	20	7.9	0	0
7/22/92	33	7.95	0	0	34	7.88	0	0	3	8.08	0	0
7/29/92	34	8.24	0	0	32	8.25	0	0	23	8.08	0	0
8/11/92	34	ND	0	0	34	ND	0	0	18	ND	0	0
8/18/92	ND	8.17	0	0	ND	8.31	0	0	2	8.37	0	0
8/26/92	33	8.08	0	0	34	8.21	0	0	22	8.18	0	0
9/2/92	32	8.07	0	0	32	8.22	0	0	14	8.23	0	0
9/12/92	ND	8.26	0	0	ND	8.18	0	0	10	8.29	0	0
10/7/92	34	8.08	0	0	34	7.46	0	0	5	8.02	0	0
10/26/92	34	7.54	0	0	34	8.17	0	0	20	8.13	a	0
11/10/92	ND	ND	ND	ND	34	8.15	0	0	25	ND	ND	ND
12/14/92	ND	ND	ND	ND	34	7.86	0	0	28	ND	ND	ND
1/25/93	33	8.26	ND	ND	32	8.25	ND	ND	22	8.25	ND	ND
3/22/93	34	8.31	ND	ND	32	8.27	ND	ND	10	8.31	ND	ND
5/17/93	ND	ND	ND	ND	30	8.11	0	0	0	ND	ND	ND
9/8/93	ND	ND	ND	ND	ND	ND	ND	ND	6	ND	ND	ND
Mean Value	34	8.1			33	8.1			15	8.17		

Table 3. Salinity, pH, and toxicity results for control sites 3 and 7

Station 3 water sample results

Date	Salinity n = 15	pH n = 11	Toxicity 5 min	Toxicity 15 min
6/8/92	34	ND	ND	ND
6/17/92	32	ND	0	0
6/29/92	34	ND	0	0
7/8/92	32	ND	0	0
7/15/92	34	7.94	0	0
7/22/92	34	8.22	0	0
7/29/92	34	8.22	0	0
8/11/92	34	ND	0	0
8/18/92	33	7.99	0	a
8/26/92	34	8.08	0	0
9/2/92	32	8.06	0	0
9/12/92	ND	8.23	0	0
10/7/92	32	8.06	0	0
10/26/92	34	7.79	0	0
11/10/92	ND	ND	ND	ND
12/14/92	ND	ND	ND	ND
1/25/93	33	8.25	ND	ND
3/22/93	34	8.23	ND	ND
5/17/93	ND	ND	ND	ND
9/8/93	ND	ND	ND	ND
Mean Value	33	8.09		

Station 7 water sample results

Date	Salinity n = 20	pH n = 15	Toxicity 5 min	Toxicity 15 min
6/8/92	15	ND	0	0
6/17/92	20	ND	0	0
6/29/92	18	ND	0	0
7/8/92	5	ND	0	0
7/15/92	14	7.77	0	0
7/22/92	10	7.48	a	0
7/29/92	15	7.55	0	0
8/11/92	14	ND	0	0
8/18/92	5	7.66	0	0
8/26/92	18	7.6	0	0
9/2/92	14	7.63	0	0
9/12/92	10	7.5	0	0
10/7/92	4	7.75	0	0
10/26/92	10	7.87	0	0
11/10/92	15	7.64	0	0
12/14/92	16	7.83	0	0
1/25/93	8	7.94	ND	ND
3/22/93	4	7.94	ND	ND
5/17/93	10	7.68	0	0
9/8/93	12	7.93	ND	ND
Mean Value	12	7.7		

Site 8 water sample results

Date	Salinity n = 20	pH n = 15	Toxicity 5 min	Toxicity 15 min
6/8/92	0	ND	ND	ND
6/17/92	0	ND	0	0
6/29/92	2	ND	0	0
7/8/92	0	ND	0	0
7/15/92	0	7.74	0	0
7/22/92	0	7.64	0	1+
7/29/92	4	7.62	0	0
8/11/92	0	ND	4+	4+
8/18/92	0	7.72	2+	2+
8/26/92	0	8.04	1+	2+
9/2/92	0	7.51	a	0
9/12/92	0	7.76	0	a
10/7/92	0	7.66	0	0
10/26/92	0	7.72	0	0
11/10/92	0	7.56	0	0
12/14/92	10	7.93	0	0
1/25/93	0	7.56	0	0
3/22/93	0	7.75	0	0
5/17/93	0	7.64	0	0
9/8/93	0	7.63	3+	3+
Mean Value	0.8	7.7		

Site 9 water sample results

Date	Salinity n = 20	pH n = 15	Toxicity 5 min	Toxicity 15 min
6/8/92	15	ND	0	0
6/17/92	18	ND	0	0
6/29/92	2	ND	3+	4+
7/8/92	22	ND	0	0
7/15/92	14	7.38	0	0
7/22/92	12	7.66	c	0
7/29/92	15	7.78	0	0
8/11/92	14	ND	0	0
8/18/92	18	7.91	0	0
8/26/92	14	7.93	0	0
9/2/92	25	7.83	0	0
9/12/92	18	7.56	0	0
10/7/92	8	7.2	0	0
10/26/92	15	7.77	0	0
11/10/92	24	7.73	0	0
12/14/92	20	7.82	0	0
1/25/93	12	7.66	0	0
3/22/93	8	7.56	0	0
5/17/93	0	7.62	b	0
9/8/93	26	7.9	0	0
Mean Value	14	7.72		

Salinity, pH and toxicity results for Honolulu 2000 storm drain stations 10, 11, 12

	Site 10 water sample results				Site 11 water sample results				Site 12 water sample results			
	Salinity	pH	Toxicity		Salinity	pH	Toxicity		Salinity	pH	Toxicity	
Date	n = 20	n = 15	5 min	15 min	n = 20	n = 15	5 min	15 min	n = 20	n = 15	5 min	15 min
6/8/92	18	ND	ND	ND	12	ND	0	0	15	ND	0	0
6/17/92	27	ND	0	0	24	ND	0	0	20	ND	0	0
6/29/92	24	ND	0	0	24	ND	0	0	18	ND	a	a
7/8/92	10	ND	b	c	6	ND	0	0	5	ND	0	0
7/15/92	24	7.76	0	0	20	7.59	0	0	14	7.77	0	0
7/22/92	5	7.84	0	0	3	7.67	0	0	10	7.48	a	a
7/29/92	18	7.73	0	0	23	7.7	0	0	15	7.55	0	0
8/11/92	20	ND	0	0	18	ND	0	0	14	ND	a	0
8/18/92	4	7.96	0	0	2	7.92	0	0	5	7.66	a	0
8/26/92	20	7.9	0	0	22	7.83	0	0	18	7.8	0	0
9/2/92	20	7.77	0	0	14	7.67	0	0	14	7.63	0	0
9/12/92	14	7.33	0	0	10	7.48	0	0	10	7.5	0	0
10/7/92	5	7.46	0	0	5	7.62	0	0	4	7.75	0	0
10/26/92	18	7.54	0	0	20	7.61	0	0	10	7.67	0	0
11/10/92	22	7.74	0	0	25	7.82	0	0	15	7.64	0	0
12/14/92	28	7.85	0	0	26	7.86	0	0	16	7.83	0	0
1/28/93	16	7.99	0	0	22	7.94	0	0	8	7.94	0	0
3/22/93	14	7.85	0	0	10	7.84	0	0	4	7.94	0	0
5/17/93	6	7.65	0	0	0	7.75	0	0	10	7.68	0	0
9/8/93	10	7.87	0	0	6	7.8	0	0	12	7.93	0	0
Mean Value	16	7.74			15	7.74			12	7.7		

Table 6. Toxicity results for the Kapahulu storm drain sediment eluate (elutriate) samples.

Site	Date	Time	EC50 (%)	Toxicity Ranking
8	8/18/92	5 min.	>100	0
		15 min.	>100	0
	11/10/92	5 min.	>100	0
		15 min.	>100	0
	12/14/92	5 min.	>100	0
		15 min.	>100	0
	1/25/93	5 min.	>100	0
		15 min.	>100	0
	3/22/93	5 min.	>100	0
		15 min.	>100	0
	5/17/93	5 min.	>100	0
		15 min.	>100	0
9	8/18/92	5 min.	>100	0
		15 min.	>100	0
	11/10/92	5 min.	>100	0
		15 min.	>100	0
	12/14/92	5 min.	>100	0
		15 min.	>100	0
	1/25/93	5 min.	ND	ND
		15 min.	ND	ND
	3/22/93	5 min.	ND	ND
		15 min.	ND	ND
	5/17/93	5 min.	ND	ND
		15 min.	ND	ND
10	8/18/92	5 min.	>100	0
		15 min.	>100	0
	11/10/92	5 min.	>100	0
		15 min.	>100	0
	12/14/92	5 min.	>100	0
		15 min.	>100	0
	1/25/93	5 min.	>100	0
		15 min.	>100	0
	3/22/93	5 min.	>100	0
		15 min.	>100	0
	5/17/93	5 min.	>100	0
		15 min.	>100	0
11	8/18/92	5 min.	>100	0
		15 min.	>100	0
	11/10/92	5 min.	>100	0
		15 min.	>100	0
	12/14/92	5 min.	>100	0
		15 min.	>100	0
	1/25/93	5 min.	>100	0
		15 min.	>100	0
	3/22/93	5 min.	>100	0
		15 min.	>100	0
	5/17/93	5 min.	b	ND
		15 min.	b	ND
12	8/18/92	5 min.	>100	0
		15 min.	>100	0
	11/10/92	5 min.	>100	0
		15 min.	>100	0
	12/14/92	5 min.	>100	0
		15 min.	>100	0
	1/25/93	5 min.	>100	0
		15 min.	>100	0
	3/22/93	5 min.	>100	0
		15 min.	>100	0
	5/17/93	5 min.	>100	0
		15 min.	>100	0

Table 7. Toxicity ranking of the Kapahulu storm drain sediment samples.

Site	Date	Time	EC50	Toxicity Ranking
8	1/25/93	5 min.	0.1418	4+
	3/22/93	5 min.	0.0253	4+
	5/17/93	5 min.	0.2159	4+
	9/8/93	5 min.	1.5085	1+
10	1/25/93	5 min.	ND	ND
	3/22/93	5 min.	3.6966	0
	5/17/93	5 min.	0.4578	4+
	9/8/93	5 min.	3.5385	0
11	1/25/93	5 min.	0.1782	4+
	3/22/93	5 min.	0.2629	4+
	5/17/93	5 min.	0.2171	4+
	9/8/93	5 min.	0.1523	4+
12	1/25/93	5 min.	ND	ND
	3/22/93	5 min.	0.1445	4+
	5/17/93	5 min.	0.4441	4+
	9/8/93	5 min.	1.3399	2+

Table 8. 95% confidence intervals for toxicity results of the Kapahulu hotel/commercial storm drain water samples.

Site	Date	Time	EC50	95% Range	95% Range Reliability
8	7/22/92	15 min.	93.5042	58.2703 -150.0428	Too large for reliability
	8/11/92	5 min.	16.2043	15.2501 - 17.2183	Acceptable
		15 min.	13.6525	12.2061 - 15.2702	Acceptable
	8/18/92	5 min.	74.0789	47.0350 - 116.6725	Too large for reliability
		15 min.	53.3523	46.2188 - 61.5868	Acceptable
	8/26/92	5 min.	92.1772	15.5568 - 546.1682	Too large for reliability
		15 min.	65.0408	43.2026 - 97.9177	Too large for reliability
	9/8/93	5 min.	45.3553	41.3786 - 45.4265	Acceptable
		15 min.	30.5404	26.7932 - 34.8115	Acceptable
9	6/29/92	5 min.	30.3041	26.9576 - 34.0660	Acceptable
		15 min.	18.8507	14.8733 - 23.8917	Too large for reliability
	7/22/92	5 min.	< 6.1875	(Exceeds Limits)	–

Table 9. 95% confidence intervals for toxicity of the Kapahulu storm drain sediment samples.

Site	Date	EC50	95% Range	95% Range Reliability
8	1/25/93	0.1418	0.1083 - 0.1857	Too Large for reliability
	3/22/93	0.0253	0.0123 - 0.0522	Too Large for reliability
	5/17/93	0.2159	0.1563 - 0.2981	Too Large for reliability
	9/8/93	1.5085	0.2979 -7.6373	Too Large for reliability
10	1/25/93	ND	ND	--
	3/22/93	3.6966	0.4489 - 30.4397	Too Large for reliability
	5/17/93	0.4578	0.1674 - 1.2519	Too Large for reliability
	9/8/93	3.5385	1.3333 - 9.3908	Too Large for reliability
11	1/25/93	0.1782	0.1440 - 0.2205	Too Large for reliability
	3/22/93	0.2629	0.1654 - 04181	Too Large for reliability
	5/17/93	0.2171	0.1308 - 0.3603	Too Large for reliability
	9/8/93	0.1523	0.0426 - 0.5442	Too Large for reliability
12	1/25/93	ND	ND	--
	3/22/93	0.1445	0.1340 - 0.1558	Acceptable
	5/17/93	0.4441	0.4151 - 0.4751	Acceptable
	9/8/93	1.3399	0.7375 - 2.4343	Too Large for reliability

Note: All readings taken at 5 minutes

EC50 values for water samples

	1		2		3		4		5	
Date	5 min	15 min	5 min	15 min	5 min	15 min	5 min	15 min	5 min	15 min
6/8/92a	>100	>100	>100	>100	ND	ND	>100	>100	>100	>100
6/17/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
6/29/92a	>100	>100	>100	a	>100	>100	>100	>100	>100	>100
7/8/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7/8/92*	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7/15/92a	a	ND	>100	>100	>100	>100	a	>100	>100	>100
7/22/92a	>100	a	>100	>100	>100	>100	>100	>100	>100	>100
7/29/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
8/11/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
8/18/92a	>100	>100	>100	>100	>100	a	>100	>100	>100	>100
8/26/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9/2/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9/12/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10/7/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10/26/92a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11/10/92a	>100	>100	>100	>100	ND	ND	ND	ND	>100	>100
12/14/92*	>100	b	>100	>100	ND	ND	ND	ND	>100	>100
1/26/93*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3/22/93*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5/17/93*	b	>100	>100	>100	ND	ND	ND	ND	>100	>100
9/8/93*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

EC50 VALUES = % Concentration

Table 10. Toxicity results for water, elutriate, and sediment samples for all sites

	6		7		8		9		10	
Date	5 min	15 min	5 min	15 min	5 min	15 min	5 min	15 min	5 min	15 min
6/8/92#	a	>100	>100	>100	ND	ND	>100	>100	>100	>100
6/17/92#	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100
6/29/92#	>100	>100	>100	>100	>100	>100	30.3041	18.8807	>100	>100
7/8/92#	>100	>100	>100	>100	>100	>100	>100	>100	b	a
7/8/92*	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7/15/92#	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7/22/92#	>100	>100	a	>100	>100	93.5042	a	>100	>100	>100
7/29/92#	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
8/11/92#	>100	>100	>100	>100	16.2043	13.8825	>100	>100	>100	>100
8/19/92#	>100	>100	>100	>100	74.0798	14.8404@ 89.3523	>100	>100	>100	>100
8/26/92#	>100	>100	>100	>100	92.1772	85.0408	>100	>100	>100	>100
9/2/92#	>100	>100	>100	>100	a	>100	>100	>100	>100	>100
9/12/92#	>100	>100	>100	>100	>100	a	>100	>100	>100	>100
10/7/92#	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10/26/92#	a	>100	>100	>100	>100	>100	>100	>100	>100	>100
11/10/92#	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100
12/14/92*	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100
1/26/93*	ND	ND	ND	ND	>100	>100	>100	>100	>100	>100
				Site 8.5	>100	>100				
3/22/93*	ND	ND	ND	ND	>100	>100	>100	>100	>100	>100
5/17/93*	ND	ND	>100	>100	>100	>100	b	>100	>100	>100
9/8/93*	ND	ND	ND	ND	43.3883	30.8404	>100	>100	>100	>100

EC50 VALUES = % Concentration

	11		12	
Date	5 min	15 min	5 min	15 min
6/8/92#	>100	>100	>100	>100
6/17/92#	>100	>100	>100	>100
6/29/92#	>100	>100	a	a
7/6/92#	>100	>100	>100	>100
7/8/92*	>100	>100	>100	>100
7/15/92#	>100	>100	>100	>100
7/22/92#	>100	>100	a	a
7/29/92#	>100	>100	>100	>100
8/11/92#	>100	>100	a	>100
8/18/92#	>100	>100	a	>100
8/26/92#	>100	>100	>100	>100
9/2/92#	>100	>100	>100	>100
9/12/92#	>100	>100	>100	>100
10/7/92#	>100	>100	>100	>100
10/26/92#	>100	>100	>100	>100
11/10/92#	>100	>100	>100	>100
12/14/92*	>100	>100	>100	>100
1/25/93*	>100	>100	>100	>100
3/22/93*	>100	>100	>100	>100
5/17/93*	>100	>100	>100	>100
9/8/93*	>100	>100	>100	>100

EC50 VALUES = % Concentration

EC50 values for elutriate samples.

	8		9		10		11		12	
Date	5 min	15 min	5 min	15 min	5 min	15 min	5 min	15 min	5 min	15 min
8/18/92#	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11/10/92#	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12/14/92*	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1/25/93*	>100	>100	ND	ND	>100	>100	>100	>100	>100	>100
	>100 d	>100 d								
3/22/93*	>100	>100	ND	ND	>100	>100	>100	>100	>100	>100
5/17/93*	>100	>100	ND	ND	>100	>100	b	b	>100	>100

EC50 values for sediment samples.

	8	9	10	11	12
Date	5 min	5 min	5 min	5 min	5 min
1/25/93	0.1419	ND	ND	0.1782	b
3/22/93	0.6283	ND	3.6666	0.2629	0.1446
5/17/93	0.2189	ND	0.4679	0.2171	0.4441
9/8/93	1.5066	ND	3.5366	0.1823	0.4441

EC50 VALUES = % Concentration

EVALUATION OF A COMMERCIALLY AVAILABLE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) KIT TO MONITOR WATER AND SOIL FOR TOXIC CHEMICALS (PESTICIDES, HERBICIDES, FUNGICIDES AND PCP)

Connie B. Paulino, Bunnie S. Yoneyama and Roger S. Fujioka

Project Completion Report KSDS-3

March 1994

PREPARED FOR
State of Hawaii
Department of Health
Contract No.: ASO Log No. 92-613
Project Period: 12 July 1993-31 December 1993
Principal Investigator: Roger S. Fujioka

WATER RESOURCES RESEARCH CENTER
University of Hawaii at Manoa
Honolulu, Hawaii 96822

I. MOTIVATION FOR STUDY

A. The Traditional Approach to Monitor Waters for Toxic Chemicals

The traditional approach to monitor waters for toxic chemicals (pesticides, herbicides, fungicide, PCP, petroleum products) requires a highly trained chemist, sophisticated and expensive equipment (gas chromatography, mass spectroscopy, high pressure liquid chromatography) as well as a special laboratory to suitably ensure that these equipment can be maintained and will function as expected. These requirements limit the number of laboratories which are capable of monitoring waters for toxic chemicals and ensure that projects requiring this need will be very expensive. These requirements also discourage the strategy of screening many suspected waters for toxic chemicals to alleviate concerns at some sites and to identify "hot spots" where more work should be done. Thus, if only the traditional approach is available, many suspected bodies of water would never be tested for the presence of toxic chemicals.

B. An Alternative and Feasible Screening Method for Toxic Chemicals

Recognizing the difficulty in using the traditional approach to monitor water for toxic chemicals, alternative and more feasible approaches have been sought. If such a simpler and less expensive method can be developed, its primary value will be its capacity to quickly screen water and soil samples from the many suspected bodies of water, most of which have never been tested, for presence of toxic chemicals. With this method, sites can be classified based on actual monitoring data as sites with (1) undetectable levels of toxic chemicals, (2) with low levels of toxic chemicals and (3) with high levels of toxic chemicals. Using this system of classifying sites, rational decisions can be made for the application of the expensive, traditional approach which has the advantage of better identifying and quantitating the toxic chemicals. Thus, the development of a more feasible approach will not replace but will complement the use of the traditional approach of testing for toxic chemicals.

The criteria for a suitable alternative method are that it (1) be relatively simple and inexpensive, (2) does not require sophisticated equipment and laboratory facilities, (3) can be run by minimally trained personnel, (4) can be completed in a short period of time and (5) can be adopted to operate under field conditions. A method fulfilling these criteria has recently been developed using the biological assay system based on the very specific reaction of antigen and antibody. In this regard, it has been well established that man and animals develop immunity to microbial pathogens by developing antibodies following an infection by the infectious agent (the antigen). An application of this method called enzyme-linked immunosorbent assay (ELISA) has been used effectively in clinical laboratories to detect and identify biological molecules such as microorganisms, toxins, blood cells and cellular proteins. In the past, this method could not be used to detect for toxic chemicals because the size of these molecules are too small to induce an antibody response in animals. This limitation was recently overcome by attaching the toxic chemical molecule to carrier molecules to increase its size and antigenicity. Using this new technology, animals will now

make antibodies to the specific structure of the toxic chemical. As a result, the very specific antigen-antibody reaction can now be used to detect for the presence of toxic chemicals.

Today, this method has progressed from the experimental phase to the development of kits which are commercially produced and made for minimally trained personnel and minimally equipped laboratories to monitor soil and waters for pesticides. Several companies are now selling some of these ELISA kits to monitor for those toxic chemical of concern (herbicides, pesticides, fungicides, petroleum compounds). After evaluating several companies, WRRC selected the Ohmicron system because this company uses antibody coated magnetized particles. This technology allows for rapid and thorough dispersion of the antibody providing for greater surface area for the antigen-antibody reaction to occur, it increases the volume of the test water to be analyzed and increases the sensitivity of the test. Moreover, the magnetized particles allow for ease of sample collection, washing and assay. Other advantages of this technology includes its adaptability to assay for toxic chemicals adsorbed to soil and to use this method under field conditions.

II. GOALS AND EXPERIMENTAL DESIGN

A. Project Goals

The first goal of this project was to evaluate the reliability and feasibility of the Ohmicron ELISA method as a means to screen water and soil for toxic chemicals.

The second goal was to apply this method to complement the toxicity monitoring component of the Kapahulu Storm Drain System Study by analyzing storm drain water samples obtained from the same sites which had been tested for bacterial content, for nutrients and for toxicity by the Microtox method.

The third goal was to apply this method to determine if toxic chemicals could be determined in typical streams in Hawaii.

B. Selection of Toxic Chemicals for Assay

ELISA kits were purchased from Ohmicron to detect toxic chemicals which are known to be used in Hawaii and which represent several broad classes of toxic chemicals. The following six compounds belonging to different families of herbicides, pesticides, fungicides and PCP were selected:

1. Atrazine, traizine family: a herbicide.
2. Benomyl/Carbendazim, benzimidazole family: a fungicide.
3. Carbaryl, carbamate family: an insecticide.
4. Chlorpyrifos: an insecticide.
5. 2,4 D, phenoxy family: a herbicide.
6. Pentachlorophenol, organochloride: a wood preservative.

Table 1 summarizes some of the basic information on the test kits and health effects of these six toxic chemicals.

III. SAMPLING SITES AND METHODOLOGY

A. Sampling Sites

The same storm drain sites 8, 9, 10, 11, and 12 previously selected in the Kapahulu Storm Drain System Study (KSDS) were selected as the primary sampling sites. The location of these sites are shown in Figure 1. In addition to these sites, water samples were obtained from a stream near an urbanized areas (Manoa Stream, Palolo Stream) and from an agricultural area (Waimanalo Stream). These samples were analyzed using the Ohmicron test kit to assess the feasibility and reliability of this method.

B. Preparation of Glassware

All glassware used in the collection and storage of samples or during the soil extraction was first treated in the following manner. The glassware was washed with soap, rinsed with tap water 5x, rinsed with 10% HCl 1x, rinsed with deionized water 10x, rinsed with methyl alcohol 1x and with distilled water 1x.

C. Collection and Storage of Samples

Water samples were collected with a rope and bucket or pole and bottle. Glass sample bottles were filled to the top. Initially, the bottle top was covered with parafilm and closed with a screw cap. Subsequent samples were placed in glass containers with ground glass stoppers or caps with a teflon coated liner. Water samples were transported to the laboratory in an ice filled cooler and stored at 8°C until use. Water was collected from storm drain sites 8, 9, 10, 11, and 12 in September and November of 1993. Detecting pollutants in low concentrations in water is often difficult due to dilution and environmental degradation. Because organic compounds sometimes adhere and accumulate on soil, they may be found in soil but not in the overlying water.

Soil samples were collected using a clean, sterile metal cup and the soil samples placed in acid washed polypropylene containers. These samples were stored at 8°C until extracted. These sediment (soil) samples were collected in November of 1993 at storm drain sites 8, 9, 10, 11, and 12. Both water and soil extracts were analyzed for toxic chemicals using the commercially available ELISA kits.

D. Use of the Ohmicron ELISA Kits

Commercially available kits are made to simplify the methodology. However, the methods as described by the company must be carried out exactly to obtain reliable results.

For each kit, there may be some slight variation in the method which is clearly described by the company. Essentially, the use of Ohmicron kit involves the following four basic steps as described in the company's literature.

1. Step 1. The sample to be analyzed is added, to a disposable test tube containing antibodies attached to magnetic particles, along with the toxic chemical to be assayed which has been labeled with an enzyme. The same toxic chemical present in the sample will compete with the labeled toxic chemical for binding sites on the antibodies. This immunological reaction occurs for 15 to 30 minutes.

2. Step 2. A magnetic field is applied to the magnetic particles which has been coated with antibodies and which will have bound the toxic chemicals in the water sample or the enzyme labeled toxic chemical. These magnetized particles are held to the tube wall while excess reagents are decanted. The particles are washed twice.

3. Step 3. The amount of enzyme labeled toxic chemical is determined by adding hydrogen peroxide and chromogen, generating a colored product. After a short incubation (20 minutes), the color production is stopped and stabilized by the addition of acid. Since the labeled toxic chemical was in competition with toxic chemical in the sample for the same antibody sites, the color development is proportional to the enzyme labeled toxic chemical added to the test kit and inversely proportional to the concentration of toxic chemical in the sample.

4. Step 4. The color reaction is read using a spectrophotometer and the concentration of the toxic chemical in the sample determined using a formula or using software which is part of the spectrophotometer.

One advantage of the Ohmicron test kit is that it can be adapted to analyze soils for toxic chemicals. Soils were extracted according to protocols obtained from the company. Generally, soil samples were mixed with a specific extractant in a glass flask, and shaken for the prescribed amount of time on a wrist action shaker. Some protocols also required an additional contact incubation of 16-24 hours. The extractant was separated from the soil sample by centrifugation, then diluted with an appropriate buffer so that the solvent would not interfere with the antigen-antibody reaction. The diluted extract was used in the ELISA method.

Specification for soil extraction procedures for the toxic chemicals assayed for in this study are summarized in Table 2. No approved soil extraction method for carbaryl has yet to be established. Therefore, only water samples were analyzed for this chemical.

Detection limits for each pesticide measured in water or soil is set by Ohmicron. The water detection limit is a reflection of the sensitivity of the ELISA method. For soil, the extraction procedure is another variable and therefore the soil detection limit also takes into account some of the uncertainty associated with extraction of the toxic chemical from soil.

IV. RESULTS AND DISCUSSION

A. Atrazine : a herbicide.

Water and sediment samples from Sites 8,9,10,11 and 12 were tested for atrazine on two separate days and the results summarized in Tables 3 and 4. Atrazine was detected in higher concentrations in storm water sample obtained from Site 8 (5.8 ppb) and at much lower concentrations from samples obtained from Site 11 (0.152 ppb) and from Site 12 (0.14 ppb). Atrazine was recovered from sediment samples at concentrations above the detectable range of 15 ppb from Sites 8, 10, 11 and 12 at concentrations ranging from 16 to 21 ppb.

These results indicate that atrazine is a common component of storm drain water and sediment which can be detected by the Ohmicron kit. The higher concentrations of atrazine in the storm drain water obtained from Site 8 may indicate higher use in hotel areas as compared to urbanized area or may reflect the fact that the use of this herbicide in a city environment is more likely to enter the storm drain than in urbanized area.

B. Benomyl/Carbendazim : a fungicide.

Water and soil samples from Sites 8,9,10,11 and 12 were tested for this fungicide (benomyl/carbendazine) and the results summarized in Table 5. Only one water sample (0.148 ppb) and possibly one sediment (36.75 ppb) obtained from site 8 appeared to be positive for benomyl/carbendazim. All of the sediment samples resulted in concentrations of this fungicide below the lowest detectable limit for this kit.

Although insufficient samples were analyzed, the available results suggest that the fungicide (benomyl/carbendazine) is less likely to be present in storm drain water as compared to atrazine.

C. Carbaryl: an insecticide.

Water samples from Sites 8,9,10,11 and 12 were tested for this insecticide (carbaryl) on two separate days and the results summarized in Table 6 and 7. Since there is no approved method to extract this insecticide from soil, sediment samples were not analyzed. Only water samples from Sites 8 (0.388 ppb) and from Site 9 (0.36 ppb) were positive for carbaryl. These results suggest that carbaryl is used more intensively in hotel area as compared to urbanized area or that the use of this insecticide in hotel area or is more likely to enter the storm drain area.

D. Chlorpyrifos: an insecticide.

Due to limitations in the reagents, only water samples from Sites 8,9 and 12 and soil samples from Sites 8, 9, 11 and 12 were tested for chlorpyrifos and the results summarized in Table 8. Water samples from Site 8 (0.405 ppb) and Site 12 (0.358ppb) were positive for chlorpyrifos while soil samples from Site 8 (221 ppb), Site 11 (179 ppb) and Site 12 (144

ppb) were also positive for this insecticide. The limited data available suggest that this insecticide (chlorpyrifos), can be expected to be found in water and sediment from storm drain systems.

E. 2,4 D : a herbicide.

Water and soil samples from Sites 8, 9, 10, 11, and 12 were tested for 2,4,D on two separate days and the results summarized in Table 9 and 10. Water samples from only Site 8 were definitely negative for 2,4 D whereas at least one water sample from Site 9 (0.79 ppb) and Site 12 (1.22 ppb) was definitely positive for this herbicide. Water samples from Sites 10 and 11 were either negative or indicated a level of 2,4,D just below the detectable level of this kit. At least one sediment sample from Site 8 (297 ppb), Site 10 (182 ppb) and Site 12 (285 ppb) were definitely positive for 2,4,D. However, for this kit the detectable level of 2,4,D in soil was high (150 ppb). Thus, recorded levels of 123 to 134 ppb 2,4,D in one of sediment samples from Sites 10 and 11 can only be interpreted as possibly positive for 2,4,D.

These results indicate that the sensitivity of this kit for recovering 2,4,D from soil needs to be improved. Despite this limitation, 2,4,D was detected in water and sediment from the storm drain.

F. Pentachlorophenol (PCP) : a wood preservative.

Water and sediment samples from Sites 8,9,10,11, and 12 were tested for this wood preservative (PCP) on two separate days and the results summarized in Table 11 and 12. PCP was detected in the water samples from Site 9 (0.21 ppb), Site 10 (0.342 ppb) and Site 12 (0.38 ppb) and from sediment samples from all sites ranging from 6400 to >10,000 ppb. Due to these high readings, these same sediment samples were re-extracted and analyzed for PCP again. In this confirmation assay, the levels of PCP were negative (Table 13). Ohmicron's technical service department was consulted to explain these results. No obvious explanation was available to explain these results. Ohmicron will send us more reagents to optimize this test for storm drain water.

Although there appears to be some discrepancy in the results of the Ohmicron test for PCP, the available evidence suggest that this wood preservative can be expected to be found in storm drains.

G. Atrazine Confirmation by Gas Chromatography

One way to confirm the results of these commercially available ELISA kits is to analyze the same sample using a traditional method such as gas chromatography. Thus, six sediment extracts and seven water samples analyzed by the ELISA method were subjected to flame ionization gas chromatography to confirm the presence or absence of atrazine. The same sediment extract that was used in the commercial ELISA kit was used for gas chromatography without additional purification. Two of the six sediment samples were

definitely positive for atrazine by ELISA, three were below the detection limit and therefore questionable, and one was non-detectable for atrazine.

The results of gas chromatography analysis of these same samples are summarized in Figures 2-7. Figure 2 displays the measurement of the gas chromatography for sediment from site 8 collected on 9/93. The top portion of the figure shows the sample run and the bottom is the standard run on the same day. In the standard run the peak at 3.627 corresponds to atrazine and the peak at 5.216 to ametryn. When the sample and standard chromatograms are compared, an atrazine peak is clearly seen in the sample run. Thus, gas chromatography analysis confirm the presence of atrazine in sediment from site 8.

The gas chromatography result for the sediment extract from site 10 collected on 9/93 is shown in Figure 3. The soil extract was concentrated from 8.2 ml to 2.1 ml. In the sample run, the small peak following the peak at 3.311 probably corresponds to the atrazine standard peak at 3.628. Thus, gas chromatography analysis confirm for the presence of atrazine in soil samples from Site 10.

Figures 4, 5, and 6 are the sample runs for extracts from sediments at Sites 8, 9, and 10 collected on 11/93. The results from these extracts with the Ohmicron ELISA kit were below the detection limit of atrazine in soil and therefore were questionable. By gas chromatography, the extract from site 8 shown in Figure 4 contains a peak at 3.645 which corresponds to the atrazine peak at 3.669 in the standard run. Some of the nearby peaks may represent metabolites of atrazine. The extract from site 9 contains no peak at or near 3.645 and appears negative (Figure 5). The extract from site 10 shows a small shoulder (Figure 6) which may correspond to the atrazine peak at 3.628 in the standard run.

Figure 7 displays the results of the gas chromatographic analysis of sediment extract from Site 9 which was definitely negative by ELISA. It also proved to be negative by gas chromatography. These results show that the negative results of the ELISA test can be relied on.

These results clearly show the limitation of sensitivity and quantitation of low levels of toxic chemicals when these ELISA test kits are used and the difficulty in interpretation when the results of the ELISA test are measurable but below the detectable limit of the kit. Under low level conditions and when one wants to determine the various forms of toxic chemicals present, the traditional gas chromatography method appears to be superior. The results of these comparative tests indicate the usefulness and complementation of these two methods.

H. Detection of Toxic Chemicals in Streams

Storm drains collect discharge from streams and the run-off from the community. Water and sediment samples from two streams (Manoa Stream, Palolo Stream) in an urbanized area and from one stream (Waimanalo Stream) from an agricultural and rural area were tested for toxic chemicals using the ELISA method. The results of these assays are

summarized in Table 14 and show that the prevalence of these six toxic chemicals are lower in streams than in storm drains. However, some toxic chemicals were detected in water and sediment samples from streams indicating that this ELISA test is applicable for stream samples.

V. CONCLUSION

The feasibility of using the commercially available ELISA kits for environmental monitoring is very good. The advantages of this system compared to the conventional methods are the ease of use, the cost of the tests, the ability to perform on site testing and the minimal sample processing necessary. The disadvantage of the ELISA kits is in the interpretation of measurements below the limit of detection for the kit. These results must be confirmed by traditional test methods. Another advantage of the ELISA kits is its ability to be used to analyze sediments for toxic chemicals. However, the sensitivity of the sediment assays must be improved. Finally, one must always be aware of interfering substances in natural, environmental waters.

The limited confirmation study that was done indicates good correlation between the results from commercial ELISA kit and from the traditional method using gas chromatography. In this confirmation testing, the advantage (greater sensitivity and versatility) of the gas chromatography method was demonstrated. This study also showed the value of using the ELISA test as a screening test and to use the gas chromatography as a confirmation test and to conduct more in-depth analysis where these kinds of study are required.

Although the commercially available ELISA method is easier to perform than gas chromatography or HPLC, it still requires training and good laboratory skills. Ability to work with small (µl) amounts of sample and reagents, consistent controlled test conditions, careful collection and storage sample are all important in generating reliable, accurate results.

The usefulness of the ELISA test will depend on the sensitivity of the reagents developed by the commercial companies. This is a new technology and companies producing the reagents are new. For many toxic chemicals, the reagents are not yet available. As with other technology, the sensitivity and cost of these reagents will drop when more companies enter this new field. The future application for the ELISA method to monitor environmental waters and sediments look very encouraging. Clearly, more evaluations and use of these alternative tests should be encouraged as a means to identify environmental problems and not to be limited because the traditional methods available are too complicated and too costly.

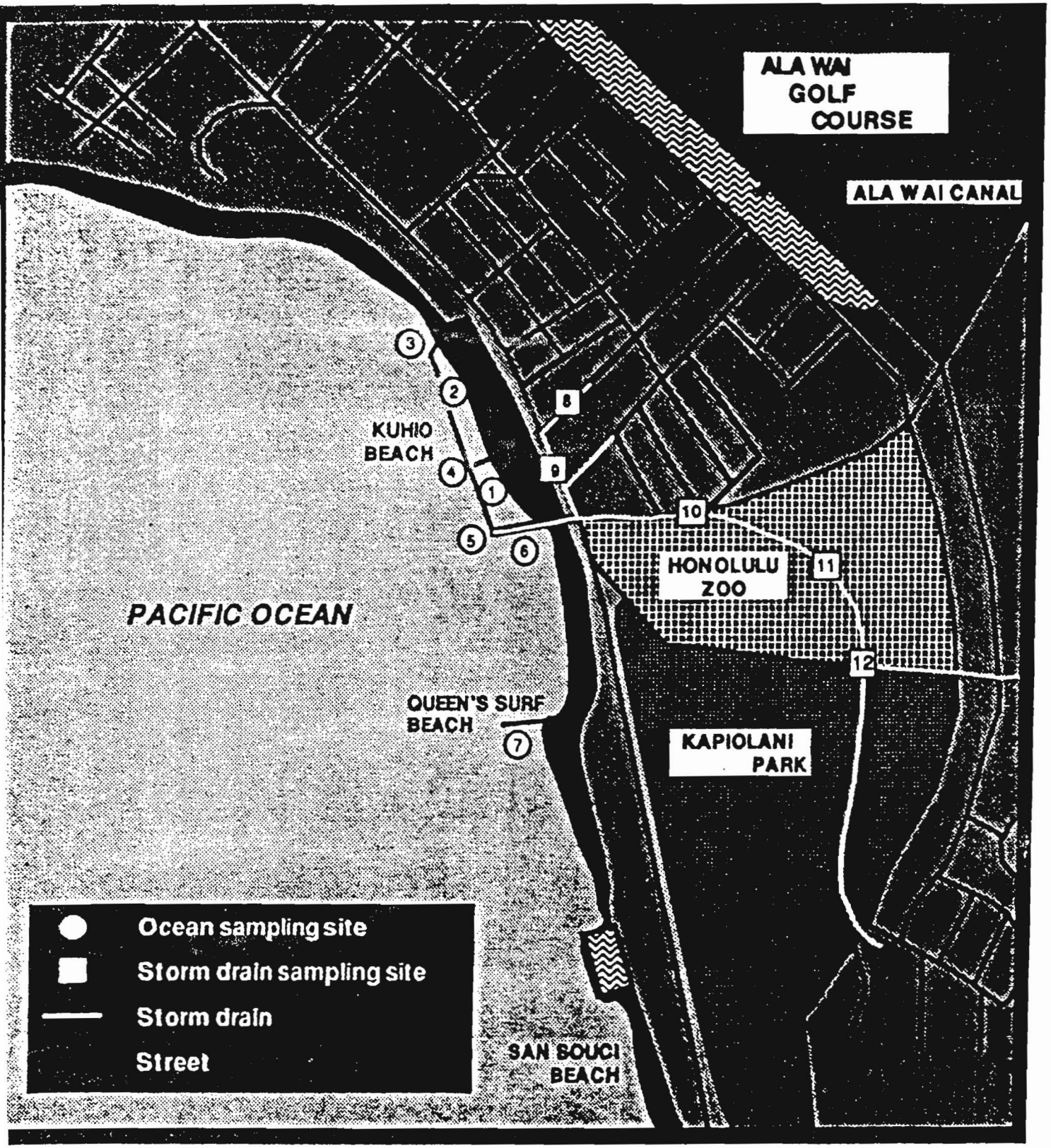


Figure 1. Sample Sites for Kapahulu Storm Drain System/Kuhio Beach Study

Table 1. Basic Information on the ELISA Test Kits and Health Effects of Six Toxic Chemicals.

Kit Name/Ohmicron Cat. #	Family	Lowest Detectable Dose (ppb)	Health Effects	Possible Sources	Maximum Contaminant Level (ppb) in Drinking Water
Atrazine/A00002	Triazine	0.046	reproductive, cardiac (e)*	Selective herbicide; used pre and post emergence for pineapple and sugar cane (a,d,f)*	3 (b)*
Benomyl or Carbendazim/ A00093	Benzimidazole	0.38	NR	Systemic fungicide (a);* used to treat pineapple crowns and sugar cane seed pieces (d,f)*	NR
Carbaryl/A00096	Carbamate	0.25	NR	Broad spectrum insecticide (a,f)*	NR
Chlorpyrifos		0.1	NR	Insecticide (a)*; available in garden stores as Dursban	NR
2,4-D/A00068	Chlorinated Phenoxy	0.7	Liver, kidney, nervous system (e)*	Selective hormone type herbicide (a)*; used for sugar cane (g,f)*; available in garden stores as Weed-B-Gon	70 (b)*
Pentachlorophenol/ A00110	Organochlorine	0.06	Possible cancer, liver, kidney (e)*	Wood preservative for fungus decay termite, or Lyctus beetle (a)*	1 (e)*

NR = Not reported
*Please refer to following page
NOTE: See appendix for other closely related metabolites detected by these kits

a) Farm Chemical Handbook, 1990.

b) National Primary Drinking Water Regulations. Synthetic Organic Chemicals and Organic Chemicals; Monitoring for Unregulated Contaminants; National Primary Drinking Water Regulations Implementation; National Secondary Drinking Water Regulations. Final Rule Fed. Reg. 56:20:3526 (January 30, 1991).

c) National Primary Drinking Water Regulations. Monitoring for Volatile Organic Chemicals; MCLG's and MCL for Aldicarb, Aldicarb Sulfoxide, Aldicarb Sulfone, Pentachlorophenol and Barium. Final Rule. Fed. Reg. 56:126:30266 (July 1. 1991).

d) Pacific Biomedical Research Center, Univ. of Hawaii, Hawaii Epidemiologic Studies Program Annual Report 11, Nov. 16, 1976 through Nov. 15, 1977 (August 1978).

e) Pontius, Frederick, Phase II Organic and Inorganic Contaminant Regulations Journal AWWA (August 1991).

f) Unpublished HSPA data.

g) Klingman, Glenn, C. and Ashton, Floyd, M., Weed Science: Principles and Practices (1975) John Wiley and Sons.

Table 2. Specifications for Soil Extraction Procedures for Each Chemical.

Chemical	Extractant (Ext.)	Ratio of Soil to Ext.	Sitting Time	Dilution Required	Conversion Factor
Atrazine	3 parts methanol: 1 part water	1 soil: 3 extractant	16 - 24 hours	1:50	150
Benomyl	1 part 0.5N NaOH: 3 parts methanol	1 soil: 3 extractant	16 - 24 hours	1:50	150
Chlorpyrifos	Prepared solvent	1 soil: 2 extractant	None	1:250	500
2,4-D	75%meth/23%water 2% acetic acid	1 soil: 3 extractant	16 - 24 hours	1:50	150
PCP	1 part 0.5N NaOH: 3 parts methanol	1 soil: 2 extractant	None	1:500	1000

Table 3. Results of Immunoassay for Atrazine in Storm Drain Water & Sediment Samples Collected September 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero(Diluent alone)	1.395	NA	0	NA	NA
Replicate	1.249				
Standard 1	1.134	87.1	0.1	NA	NA
Replicate	1.169				
Standard 2	0.622	47.05	1	NA	NA
Replicate	0.622				
Standard 3	0.323	23.87	5	NA	NA
Replicate	0.308				
Control					
[3.0 + 0.6ppb]	0.402	30.41	2.9	NA	NA
Site 8 - Water	0.309	23.37	4.68	NA	4.68
- Sediment	1.092	82.6	0.14	150	21
Site 9 - Water	1.263	95.54	NEG	NA	NEG
- Sediment	1.158	87.59	0.083	150	12.45*
Site 10 - Water	1.234	93.34	NEG	NA	NEG
- Sediment	1.117	84.49	0.128	150	19.2
Site 11 - Water	1.196	90.47	NEG	NA	NEG
- Sediment	1.137	86	0.12	150	18
Site 12 - Water	1.097	82.98	0.14	NA	0.14
- Sediment	1.157	87.52	0.088	150	13.2*

NA = Not applicable
NEG=Negative (below lowest detection limit)
Detection limit in water = 0.046ppb
Detection limit in soil = 15ppb
* = subject to cross-reactivity or cross-interference

Table 4. Results of Immunoassay for Atrazine in Storm Drain Water & Sediment Samples Collected November 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	1.126	NA	0	NA	NA
Replicate	1.188				
Standard 1	0.926	80.5	0.1	NA	NA
Replicate	0.937				
Standard 2	0.5	43.3	1	NA	NA
Replicate	0.501				
Standard 3	0.269	24	5	NA	NA
Replicate	0.266				
Control					
[3.0 ± 0.6 ppb]	0.296	25.6	3	NA	NA
Site 8 - Water	0.227	19.6	5.8	NA	5.8
- Sediment	0.97	83.8	0.088	150	13.2*
Site 9 - Water	1.023	88.4	NEG	NA	NEG
- Sediment	1.026	88.7	NEG	150	NEG
Site 10 - Water	1.02	88.2	NEG	NA	NEG
- Sediment	1.005	86.9	0.063	150	9.45*
Site 11 - Water	0.899	77.7	0.152	NA	0.152
- Sediment	0.986	85.2	0.077	150	11.55*
Site 12 - Water	0.936	80.9	0.118	NA	0.118
- Sediment	0.947	81.8	0.109	150	16.35

NA = Not applicable
NEG=Negative (below lowest detection limit)
Detection limit in water = 0.046ppb
Detection limit in soil = 15ppb
* = subject to cross reactivity or cross-interference

Table 5. Results of Immunoassay for Benomyl in Storm Drain Water & Sediment Samples Collected November 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	1.45	NA	0	NA	NA
Replicate	1.387				
Standard 1	1.121	81.2	0.25	NA	NA
Replicate	1.182				
Standard 2	0.784	57.6	1	NA	NA
Replicate	0.851				
Standard 3	0.39	26.5	5	NA	NA
Replicate	0.362				
CONTROL					
[2.5 + 0.5ppb]	0.557	39.3	2.38	NA	
Site 8 - Water	1.227	86.5	0.148	NA	0.148
- Sediment	1.147	80.9	0.245	150	36.75*
Site 9 - Water	1.277	90	0.098	NA	0.098*
- Sediment	1.326	93.5	0.075	150	11.25*
Site 10 - Water	1.307	92.1	0.083	NA	0.083*
- Sediment	1.246	87.9	0.128	150	19.2*
Site 11 - Water	1.328	93.6	0.075	NA	0.075*
- Sediment	1.239	87.3	0.135	150	20.25*
Site 12 - Water	1.32	93.1	0.078	NA	0.078*
- Sediment	1.311	92.4	0.085	150	12.75*

NA = Not applicable
Detection limit in water = 0.1ppb
Detection limit in soil =37.5ppb
* = subject to cross-reactivity or cross-interference

Table 6. Results of Immunoassay for Carbaryl in Storm Drain Water Samples Collected September 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	1.381	NA	0	NA	NA
Replicate	1.403				
Standard 1	1.162	80.39	0.4	NA	NA
Replicate	1.076				
Standard 2	0.972	67.89	1.5	NA	NA
Replicate	0.918				
Standard 3	0.569	40.19	5	NA	NA
Replicate	0.55				
CONTROL					
[2.0 + 0.4 ppb]	0.835	59.99	2.08	NA	NA
Site 8 - Water	1.299	93.32	0.228	NA	0.228*
- Sediment	ND	NA	NA	NA	NA
Site 9 - Water	1.506	108.18	NEG	NA	NEG
- Sediment	ND	NA	NA	NA	NA
Site 10 - Water	1.451	104.24	NEG	NA	NEG
- Sediment	ND	NA	NA	NA	NA
Site 11 - Water	1.468	105.46	NEG	NA	NEG
- Sediment	ND	NA	NA	NA	NA
Site 12 - Water	1.388	99.71	NEG	NA	NEG
- Sediment	ND	NA	NA	NA	NA

NA=Not applicable
NEG=Negative (below lowest detection limit)
ND=Not done
Detection limit in water = 0.25ppb
* = subject to cross-reactivity or cross-interference

Table 7. Results of Immunoassay for Carbaryl in Storm Drain Water Samples Collected November 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	0.925	NA	0	NA	NA
Replicate	0.969				
Standard 1	0.797	84.3	0.4	NA	NA
Replicate	0.8				
Standard 2	0.642	67.05	1.5	NA	NA
Replicate	0.628				
Standard 3	0.382	42.03	5	NA	NA
Replicate	0.414				
CONTROL					
[2.0 ± 0.4ppb]	0.602	63.6	1.75	NA	NA
Site 8 - Water	0.83	87.6	0.388	NA	0.388
- Sediment	ND	NA	NA	NA	NA
Site 9 - Water	0.841	88.8	0.36	NA	0.36
- Sediment	ND	NA	NA	NA	NA
Site 10 - Water	1.006	106.2	NEG	NA	NEG
- Sediment	ND	NA	NA	NA	NA
Site 11 - Water	0.972	102.6	NEG	NA	NEG
- Sediment	ND	NA	NA	NA	NA
Site 12 - Water	0.975	102.9	NEG	NA	NEG
- Sediment	ND	ND	NA	NA	NA

NA=Not applicable
NEG=Negative (below lowest detection limit)
ND=Not done
Detection limit in water = 0.25ppb

Table 8. Results of Immunoassay for Chlorpyrifos in Storm Drain Water & Sediment Samples Collected November 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	0.672	NA	0	NA	NA
Replicate	ND	NA	NA	NA	NA
Standard 1	0.587	87.4	0.22	NA	NA
Replicate	ND	NA	NA	NA	NA
Standard 2	0.27	40.2	1	NA	NA
Replicate	ND	NA	NA	NA	NA
Standard 3	0.174	25.9	3	NA	NA
Replicate	ND	NA	NA	NA	NA
CONTROL [1.8 + 0.36ppb]	0.228	33.9	1.68	NA	NA
Site 8 - Water	0.553	82.3	0.405	NA	0.405
- Sediment	0.532	79.17	0.442	500	221
Site 9 - Water	0.679	101	NEG	NA	NEG
- Sediment	0.688	102.4	NEG	500	NEG
Site 10 - Water	ND	NA	NA	NA	NA
- Sediment	ND	NA	NA	500	NA
Site 11 - Water	ND	NA	NA	NA	NA
- Sediment	0.566	84.2	0.358	500	179
Site 12 - Water	0.566	84.2	0.358	NA	0.358
- Sediment	0.59	87.79	0.288	500	144

NA=Not applicable
NEG=Negative (below lowest detection limit)
ND=Not done
Detection limit in water = 0.1ppb
Detection limit in soil = 100ppb

Table 9. Results of Immunoassay for 2,4-D in Storm Drain Water & Sediment Samples Collected September 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	1.341	NA	0	NA	NA
Replicate	1.363				
Standard 1	1.152	85.95	1	NA	NA
Replicate	1.172				
Standard 2	0.784	57.17	10	NA	NA
Replicate	0.762				
Standard 3	0.513	36.06	50	NA	NA
Replicate	0.462				
Control [35 + 7 ppb]	0.572	42.31	31	NA	NA
Site 8 - Water	1.291	95.48	NEG	NA	NEG
- Sediment	1.031	76.26	1.98	150	297
Site 9 - Water	1.174	86.83	0.79	NA	0.79
- Sediment	1.17	86.54	0.82	150	123*
Site 10 - Water	1.258	93.05	NEG	NA	NEG
- Sediment	1.126	83.28	1.21	150	181.5
Site 11 - Water	1.245	92.08	NEG	NA	NEG
- Sediment	1.158	85.65	0.89	150	133.5*
Site 12 - Water	1.129	83.51	1.22	NA	1.22
- Sediment	1.041	76.99	1.9	150	285

NA = Not applicable
NEG=Negative (below lowest detection limit)
Detection limit in water = 0.7 ppb
Detection limit in soil = 150ppb
* = subject to cross-reactivity or cross-interference

Table 10. Results of Immunoassay for 2,4-D in Storm Drain Water & Sediment Samples Collected November 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	1.434	NA	0	NA	NA
Replicate	1.467				
Standard 1	1.254	84	1	NA	NA
Replicate	1.184				
Standard 2	0.819	55.5	10	NA	NA
Replicate	0.792				
Standard 3	0.51	34.9	50	NA	NA
Replicate	0.502				
Control					
[35 + 7 ppb]	0.629	43.4	30	NA	NA
Site 8 - Water	1.573	108.4	NEG	NA	NEG
- Sediment	1.243	85.7	0.8	150	120*
Site 9 - Water	1.299	89.6	0.57	NA	0.57*
- Sediment	1.364	94	NEG	150	NEG
Site 10 - Water	1.288	88.7	0.63	NA	0.63*
- Sediment	1.359	93.7	NEG	150	NEG
Site 11 - Water	1.282	88.4	0.65	NA	0.65*
- Sediment	1.301	89.7	0.55	150	82.5*
Site 12 - Water	1.255	86.5	0.76	NA	0.76
- Sediment	1.366	94.2	NEG	150	NEG

NA = Not applicable
NEG=Negative (below lowest detection limit)
Detection limit in water = 0.7ppb
Detection limit in soil = 150ppb
* = subject to cross-reactivity or cross-interference

Table 11. Results of Immunoassay for PCP in Storm Drain Water & Sediment Samples Collected September 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	1.129	NA	0	NA	NA
Replicate	1.089				
Standard 1	0.97	85.89	0.1	NA	NA
Replicate	0.935				
Standard 2	0.525	45.76	2	NA	NA
Replicate	0.49				
Standard 3	0.299	32.15	10	NA	NA
Replicate	0.414				
CONTROL					
[1.0 + 0.3ppb]	0.616	55.55	1.38	NA	NA
Site 8 - Water	1.207	108.84	NEG	NA	NEG
- Sediment	1.102	99.34	NEG	1000	NEG
Site 9 - Water	1.033	93.15	NEG	NA	NEG
- Sediment	1.188	107.2	NEG	1000	NEG
Site 10 - Water	1.088	98.12	NEG	NA	NEG
- Sediment	1.146	103.34	NEG	1000	NEG
Site 11 - Water	1.127	101.62	NEG	NA	NEG
- Sediment	1.126	101.53	NEG	1000	NEG
Site 12 - Water	1.176	106.04	NEG	NA	NEG
- Sediment	1.184	106.76	NEG	1000	NEG

NA = Not applicable
NEG=Negative (below lowest detection limit)
Detection limit in water = 0.1ppb
Detection limit in soil =100ppb

Table 12. Results of Immunoassay for PCP in Storm Drain Water & Sediment Samples Collected November 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	1.081	NA	0	NA	NA
Replicate	1.039				
Standard 1	0.959	91.4	0.1	NA	NA
Replicate	0.979				
Standard 2	0.531	50.1	2	NA	NA
Replicate	NA				
Standard 3	0.316	29.8	10	NA	NA
Replicate	NA				
CONTROL [1.0 + 0.3ppb]	NA	NA	NA	NA	NA
Site 8 - Water	1.006	94.9	NEG	NA	NEG
- Sediment	0.148	14	>10	1000	>10,000
Site 9 - Water	0.893	84.2	0.21	NA	0.21
- Sediment	0.186	17.5	>10	1000	>10,000
Site 10 - Water	0.84	79.2	0.342	NA	0.342
- Sediment	0.341	32.2	7.4	1000	7400
Site 11 - Water	1.135	107.1	NEG	NA	NEG
- Sediment	0.334	31.5	7.55	1000	7550
Site 12 - Water	0.826	78	0.38	NA	0.38
- Sediment	0.361	34.1	6.4	1000	6400

NA = Not applicable
NEG=Negative (below lowest detection limit)
Detection limit in water = 0.1ppb
Detection limit in soil =100ppb

Table 13. Results of Immunoassay of Re-Test of PCP in Storm Drain Water & Sediment Samples Collected November 1993.

Sample	Assay Result (Absorbance)	%B/Bo	Conc. (ppb)	Conversion Factor	Final Conc. (ppb)
Zero (Diluent alone)	1.001	NA	0	NA	NA
Replicate	0.966				
Standard 1	0.878	87.3	0.1	NA	NA
Replicate	0.839				
Standard 2	0.464	44.5	2	NA	NA
Replicate	0.412				
Standard 3	0.282	28.9	10	NA	NA
Replicate	0.287				
CONTROL					
[1.0 + 0.3ppb]	0.723	73.5	0.57	NA	NA
Site 8 - Water	ND	NA	NA	NA	NA
- Sediment	0.926	94.2	0.088	1000	88*
Site 9 - Water	ND	NA	NA	NA	NA
- Sediment	1.01	102.7	NEG	1000	NEG
Site 10 - Water	ND	NA	NA	NA	NA
- Sediment	0.982	99.8	NEG	1000	NEG
Site 11 - Water	ND	NA	NA	NA	NA
- Sediment	0.982	99.8	NEG	1000	NEG
Site 12 - Water	ND	NA	NA	NA	NA
- Sediment	0.993	100.9	NEG	1000	NEG

NA=Not applicable
NEG=Negative (below lowest detection limit)
ND=Not done
Detection limit in water = 0.1ppb
Detection limit in soil =100ppb

Table 14. Levels of Contaminants in Water & Sediment Samples Collected September & November 1993.

Sample		Date	Atrazine DL(W)=0.048ppb DL(S)=15ppb	Benomyl (ppb) DL(W)=0.1ppb DL(S)=37.5ppb	Carbaryl (ppb) DL(W)=0.25ppb	Chlorpyrifos (ppb DL(W)=0.1ppb DL(S)=100ppb	2, 4 - D (ppb) DL(W)=0.7ppb DL(S)=150ppb	PCP (ppb) DL(W)=0.06 DL(S)=100ppb
Manoa	- Water	Sep-93	NEG	ND	NEG	ND	NEG	NEG
		Nov-93	NEG	ND	NEG	ND	NEG	NEG
	- Sediment	Sep-93	ND	ND	ND	ND	ND	ND
		Nov-93	13.5*	18*	ND	ND	NEG	NEG
Palolo	- Water	Sep-93	NEG	ND	NEG	ND	NEG	NEG
		Nov-93	NEG	ND	NEG	ND	NEG	NEG
	- Sediment	Sep-93	ND	ND	ND	ND	ND	ND
		Nov-93	11.25*	16.8*	ND	ND	NEG	NEG
Waim.	- Water	Sep-93	NEG	ND	NEG	ND	NEG	NEG
		Nov-93	NEG	NEG	0.463	ND	NEG	NEG
	- Sediment	Sep-93	ND	ND	ND	ND	ND	ND
		Nov-93	NEG	NEG	ND	ND	NEG	>10,000

ND=Not done
NEG=Negative (below lowest detection limit)
* = subject to cross-reactivity or cross-interference
DL(W) = Detection limit in water
DL(S)= Detection limit in soil

Table 15. Presence/Absence of Contaminants in Water & Sediment Samples Collected September & November 1993.

Sample		Date	Atrazine	Benomyl	Carbaryl	Chlorpyrifos	2, 4 - D	PCP
Manoa	- Water	Sep-93	-	ND	-	ND	-	-
		Nov-93	-	ND	-	ND	-	-
	- Sediment	Sep-93	ND	ND	ND	ND	ND	ND
		Nov-93	+/-	+/-	ND	ND	-	-
Palolo	- Water	Sep-93	-	ND	-	ND	-	-
		Nov-93	-	ND	-	ND	-	-
	- Sediment	Sep-93	ND	ND	ND	ND	ND	ND
		Nov-93	+/-	+/-	ND	ND	-	-
Waim.	- Water	Sep-93	-	ND	-	ND	-	-
		Nov-93	-	-	+	ND	-	-
	- Sediment	Sep-93	ND	ND	ND	ND	ND	ND
		Nov-93	-	-	ND	ND	-	+

+/- = Below lowest detection limit
ND=Not done

Site 8 Collected in September 1993.

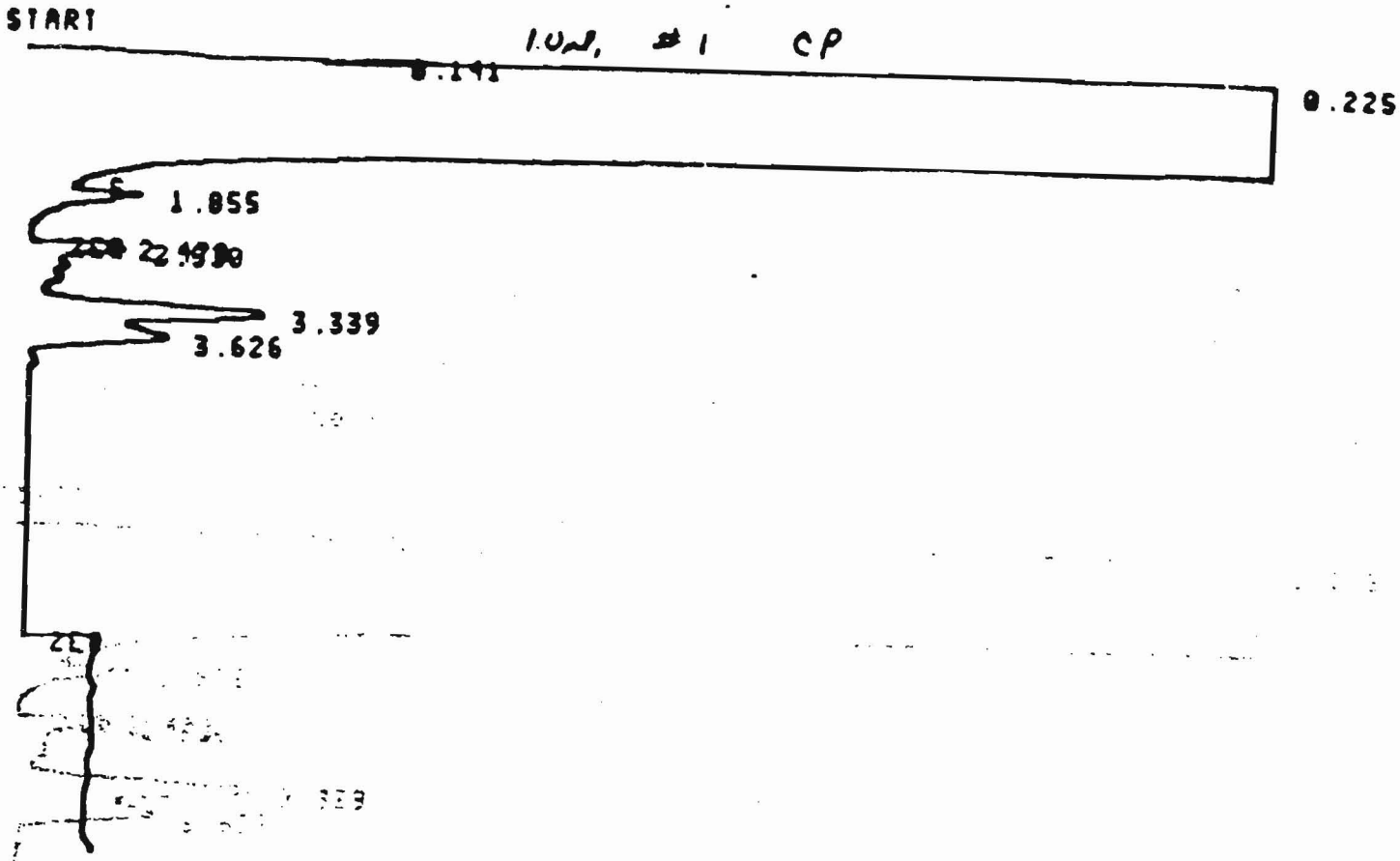


Figure 2b. Gas Chromatogram of Atrazine Standard.

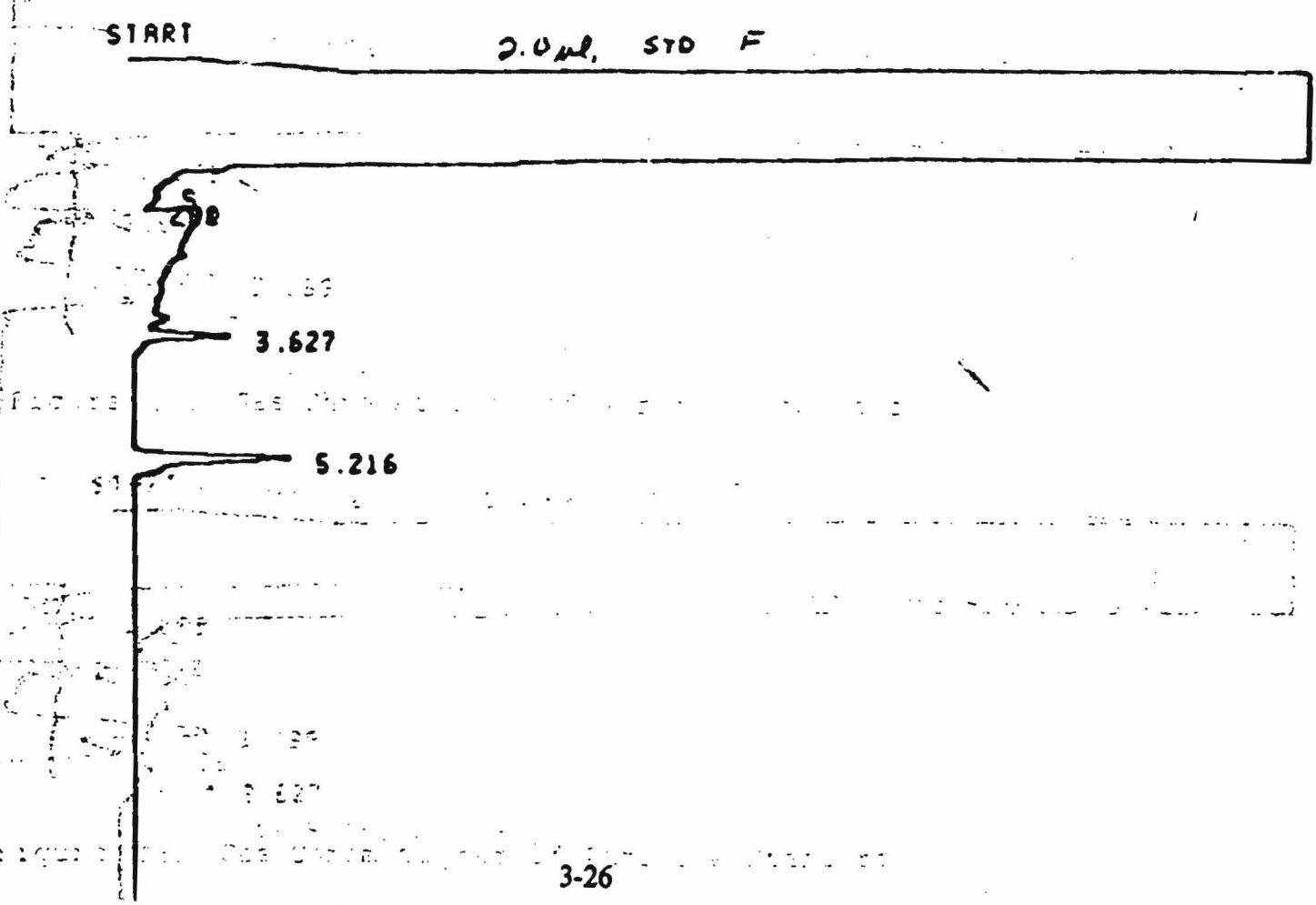


Figure 3a. Gas Chromatogram of Extract from Soil at Site 10 Collected in September 1993.

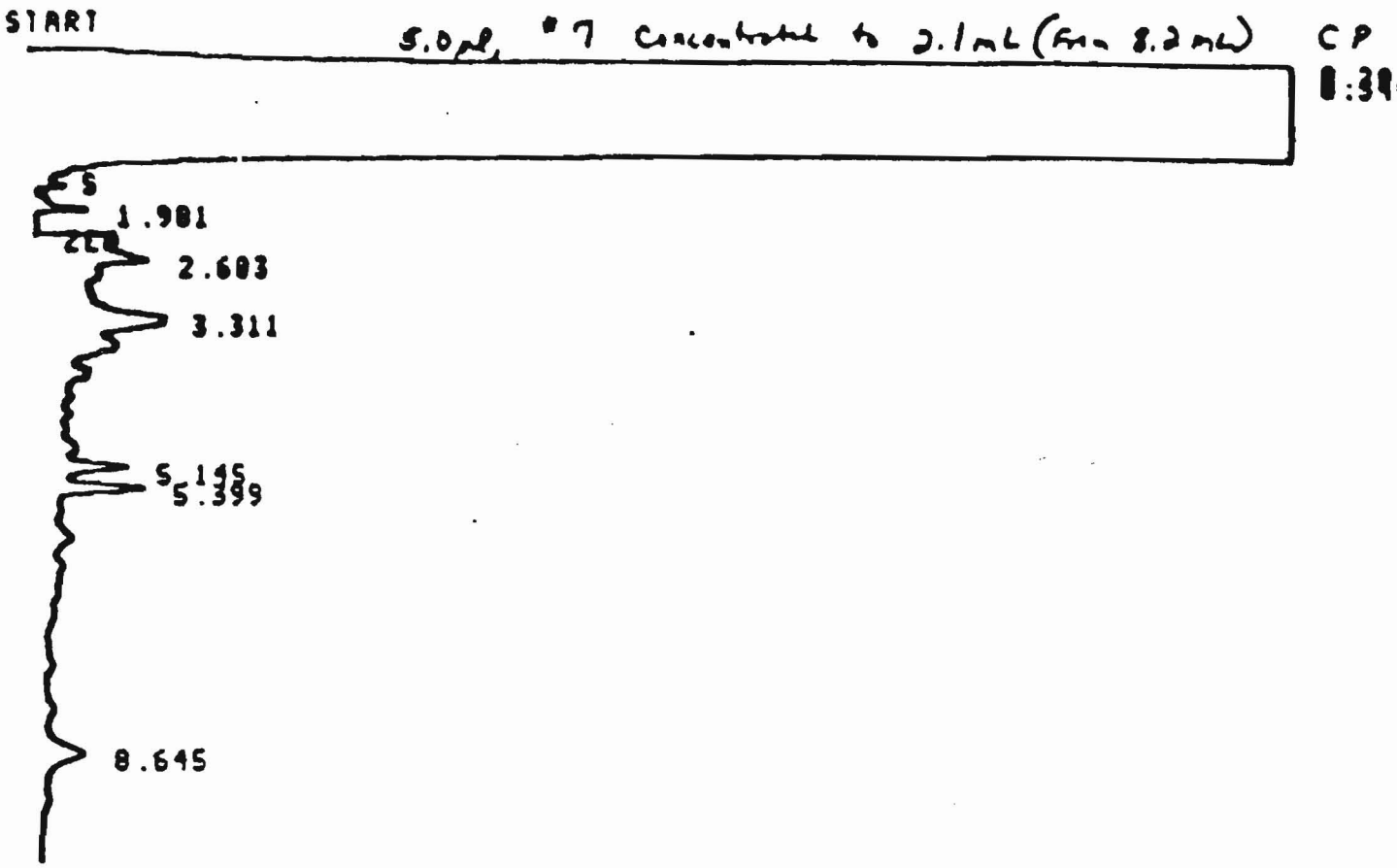


Figure 3b. Gas Chromatogram of Atrazine Standard.

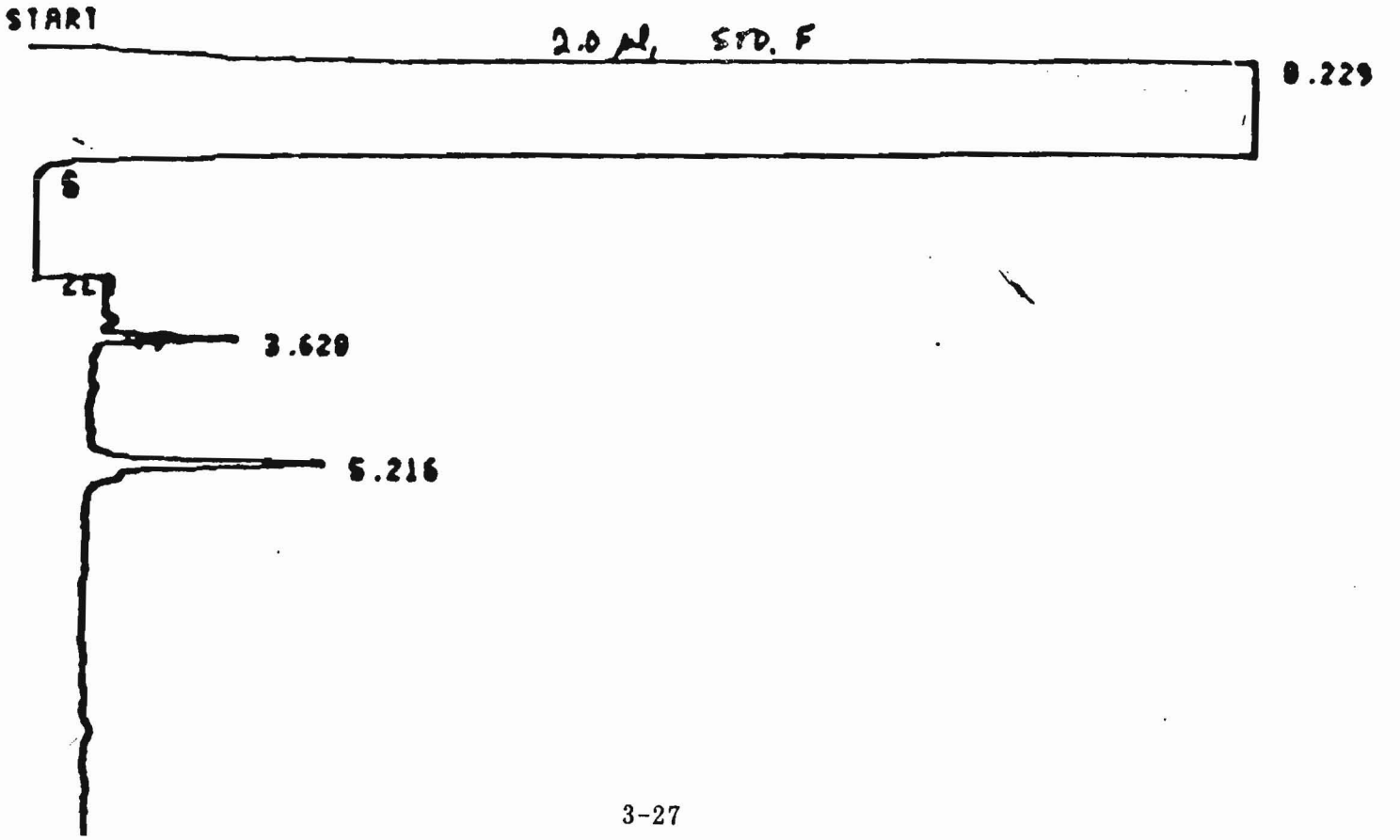


Figure 4a. Gas Chromatogram of Extract from Soil at Site 8 Collected in November 1993.

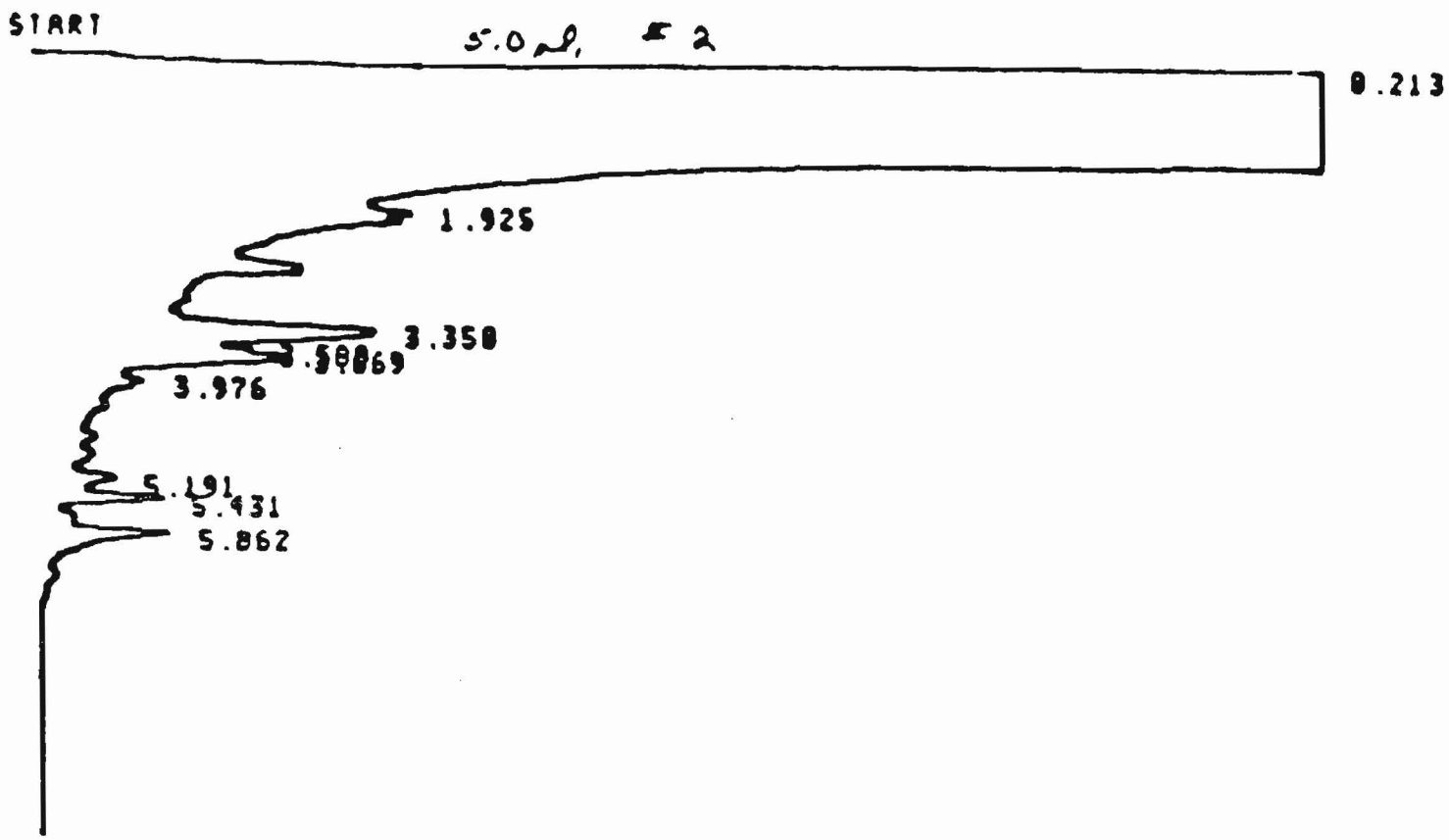
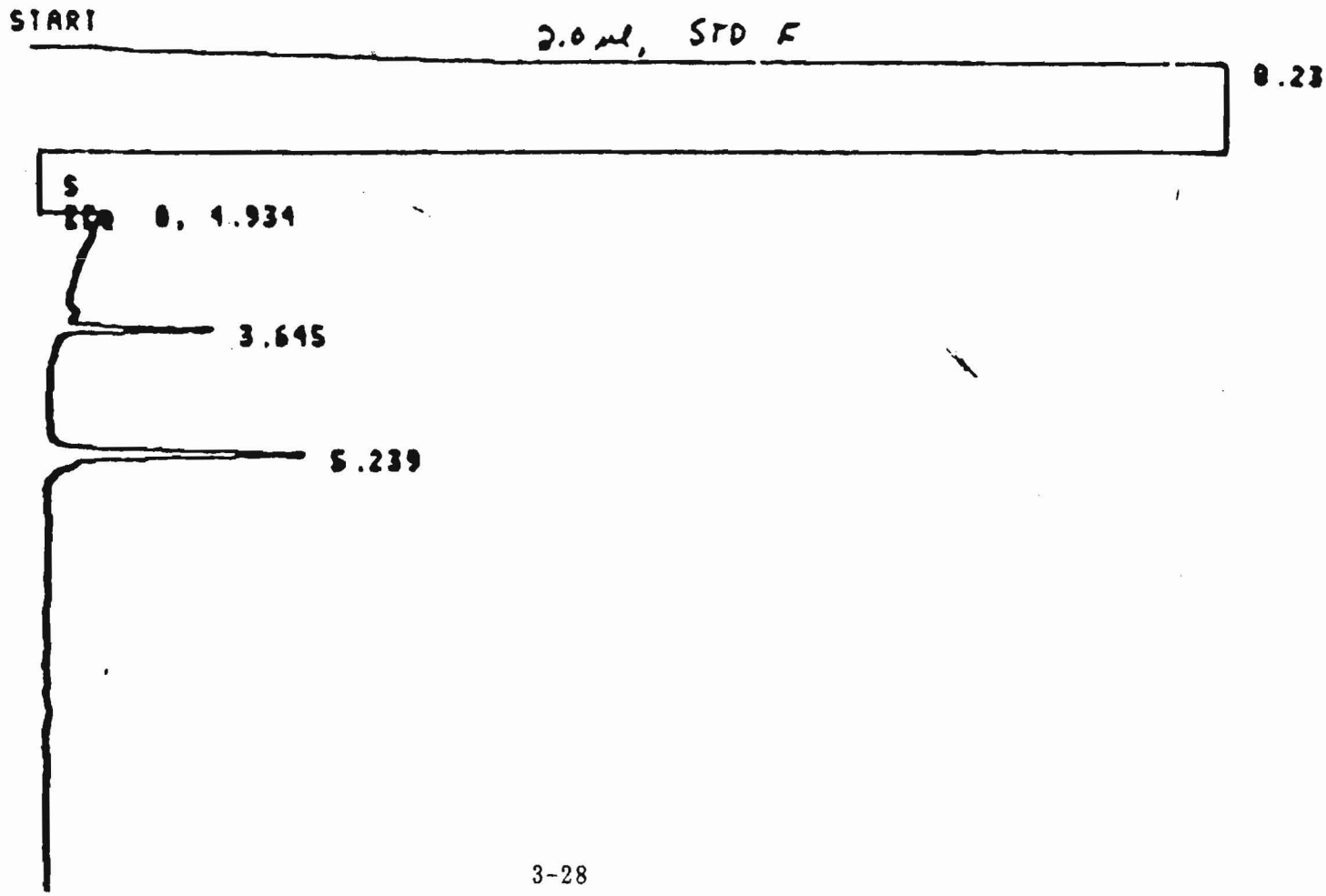


Figure 4b. Gas Chromatogram of Atrazine Standard.



Site 9 Collected in September 1993.

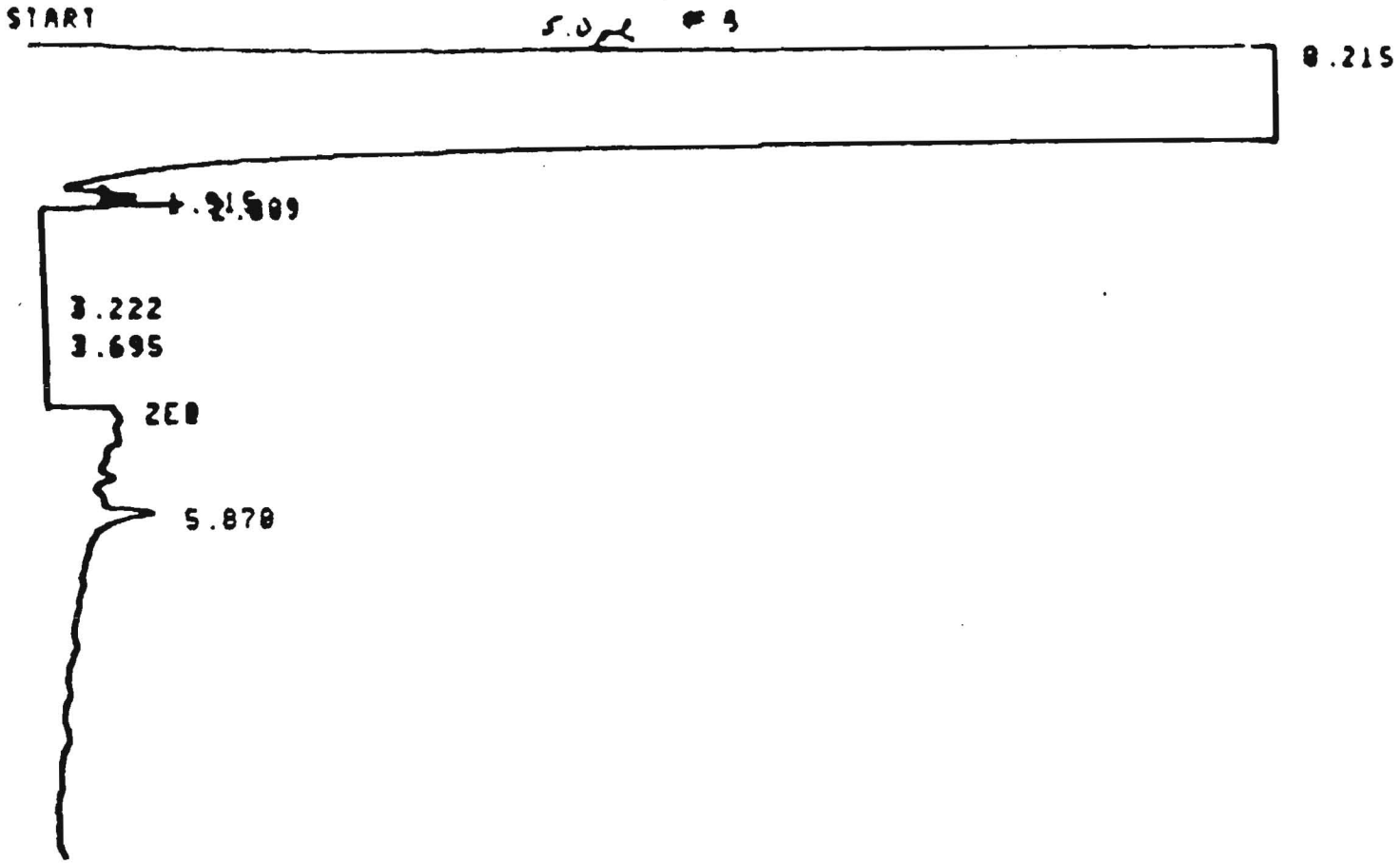
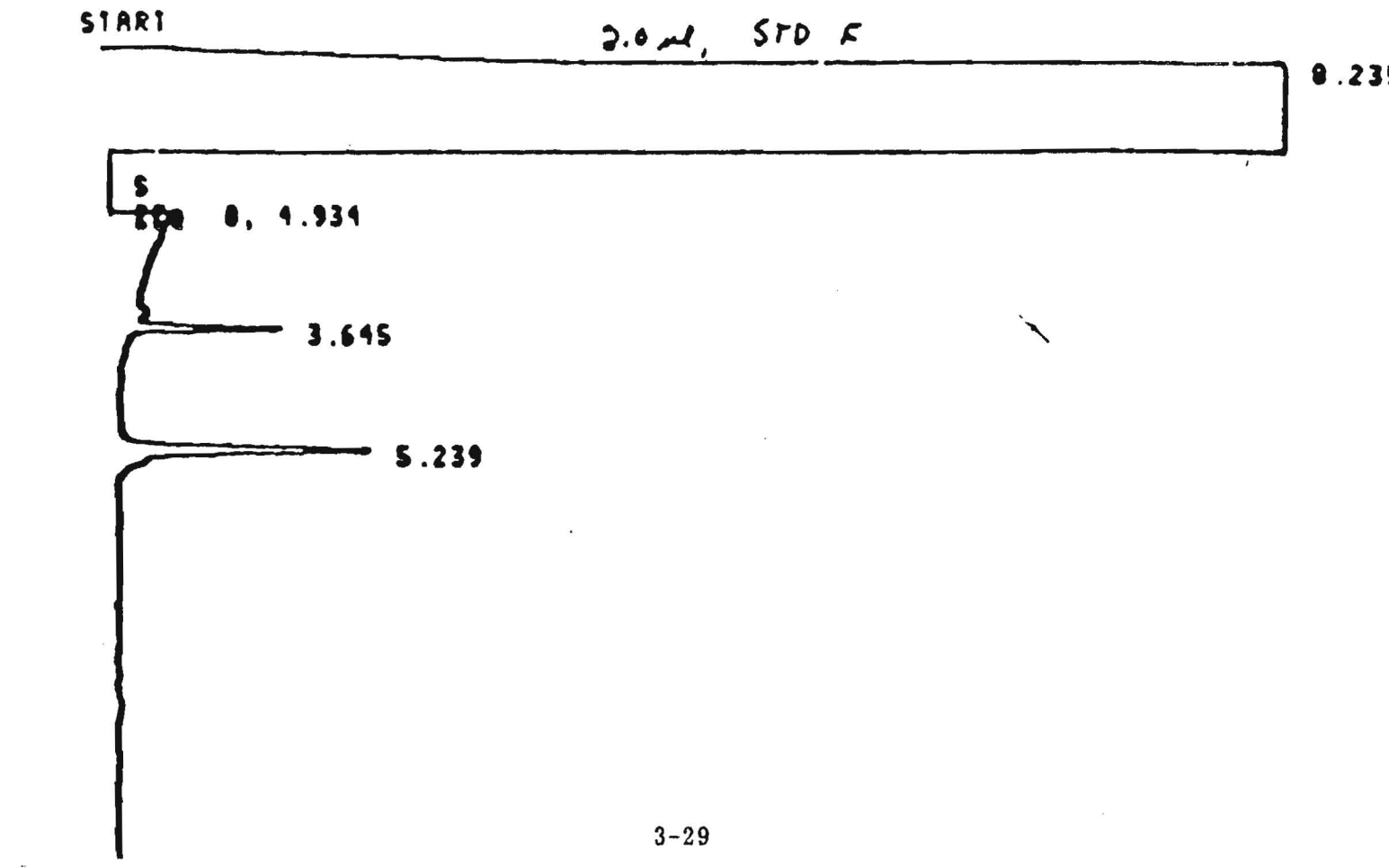


Figure 5b. Gas Chromatogram of Atrazine Standard.



Site 10 Collected in November 1993.

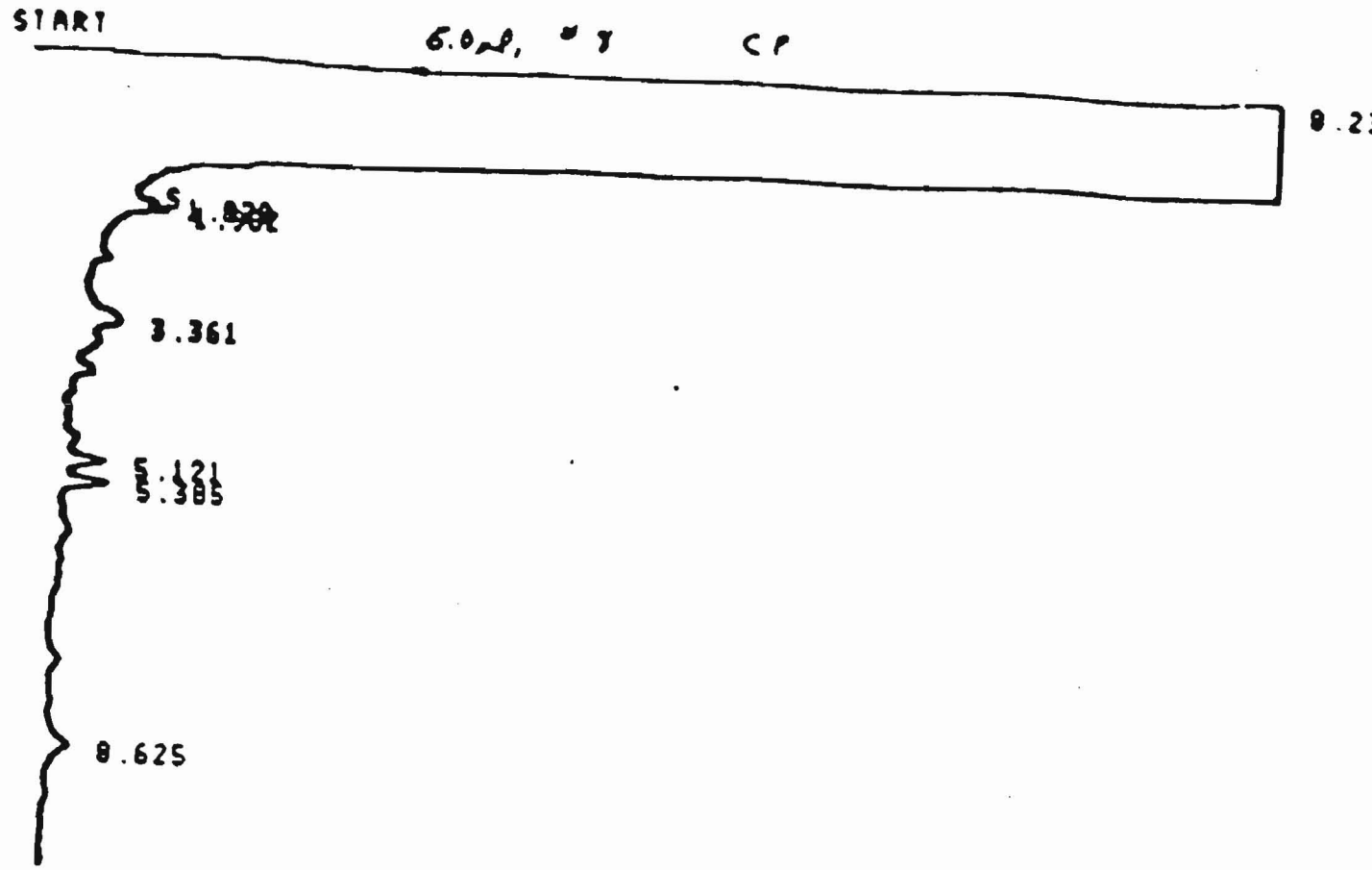


Figure 6b. Gas Chromatogram of Atrazine Standard.

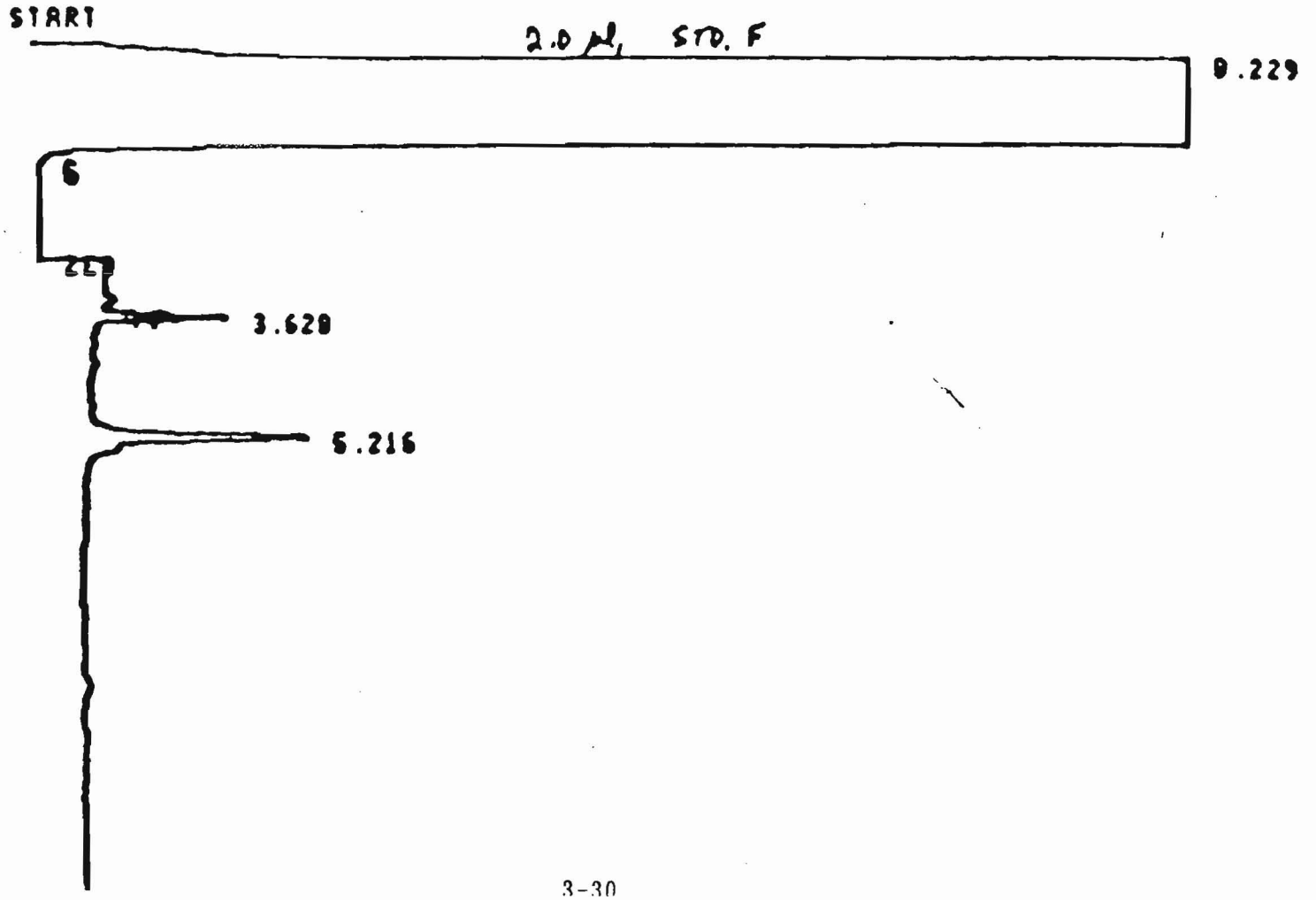


Figure [illegible]
Site 9 Collected in November 1993.

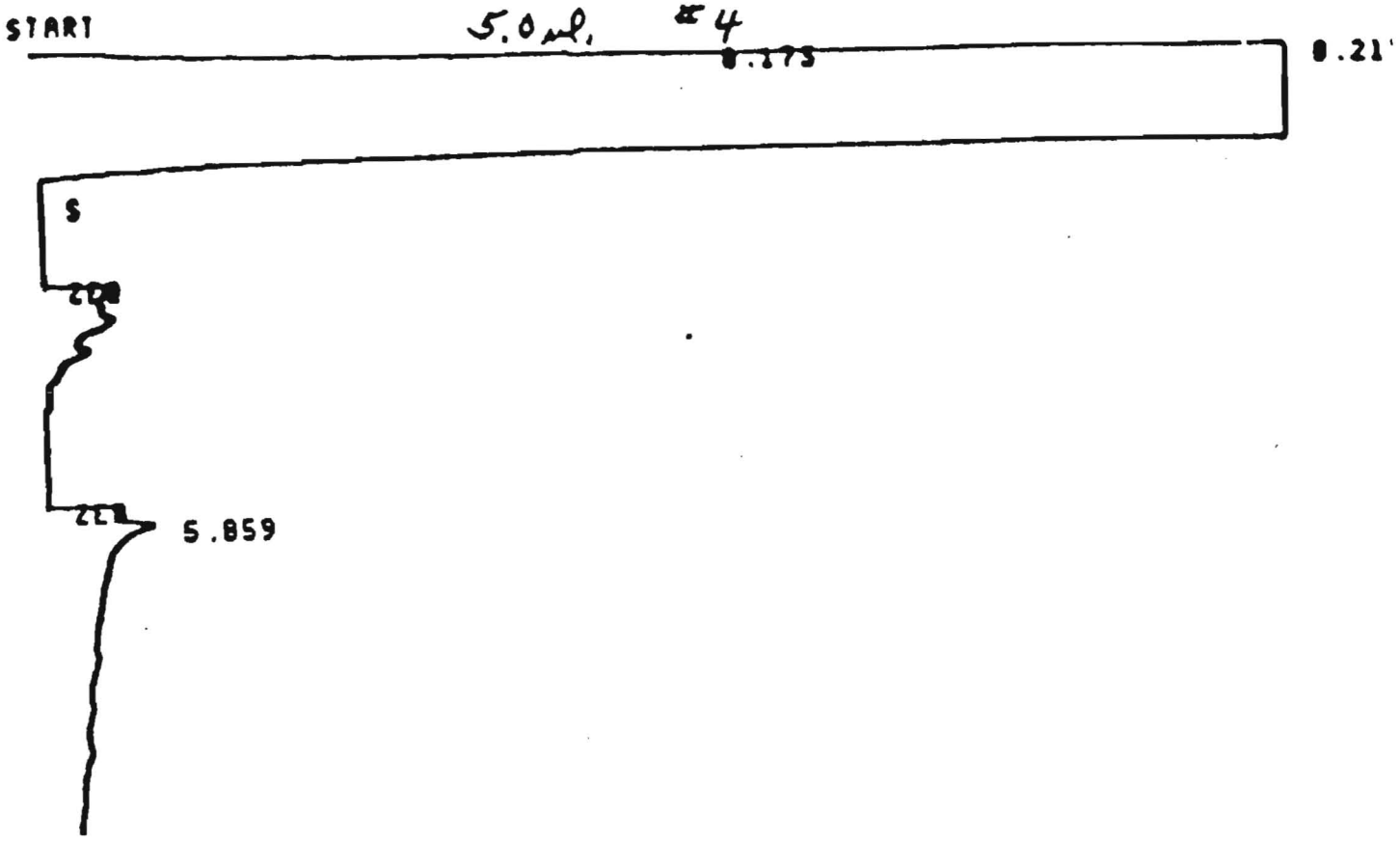
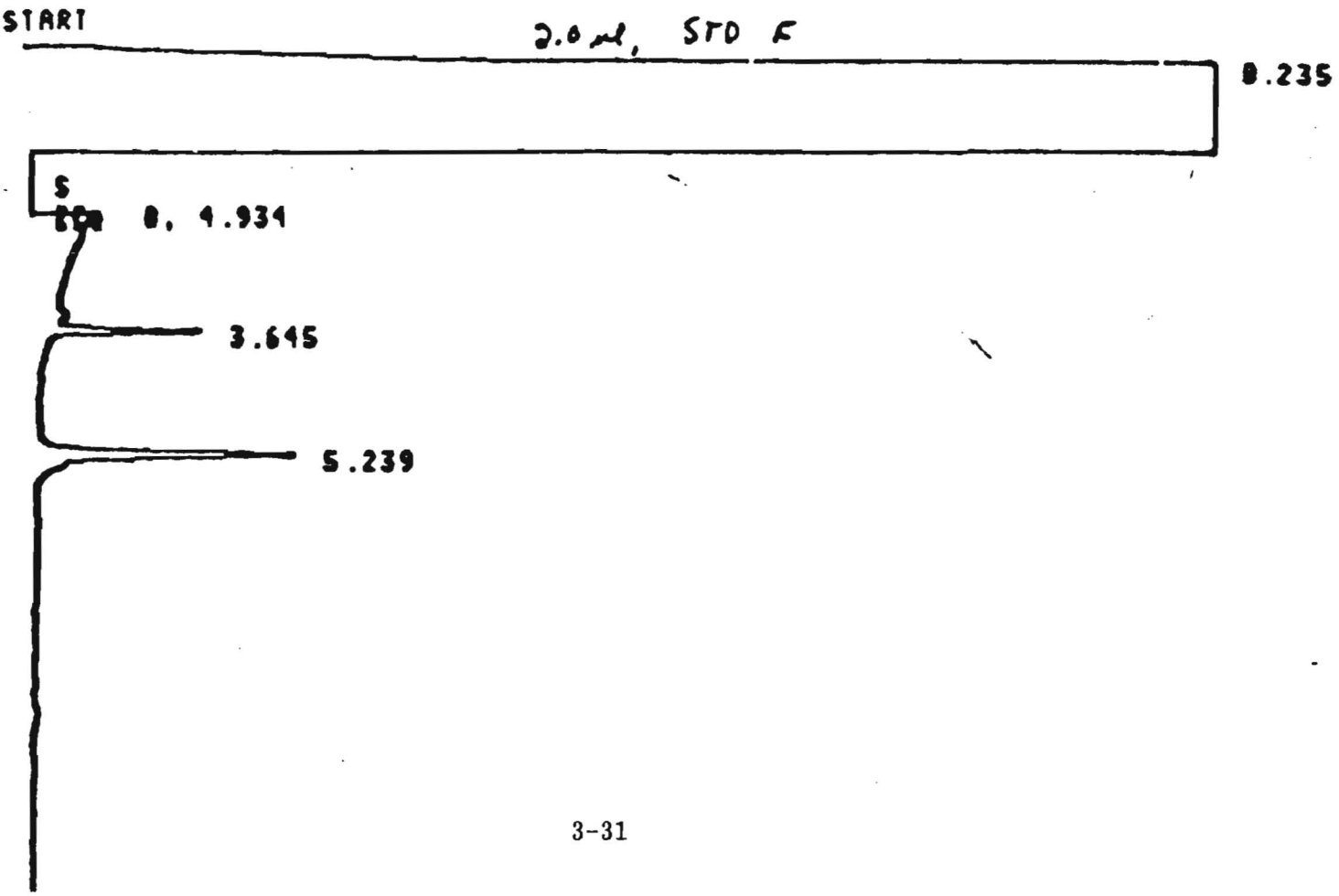


Figure 7b. Gas Chromatogram of Atrazine Standard.



APPENDIX

Ohmicron Company's publication on the precision, sensitivity and cross-reactivity of the various toxic chemicals used in the ELISA test kits

- **Selective**
- **Accurate**
- **Efficient**
- **Sensitive**
- **Clean**

RaPID Assays apply the principles of enzyme linked immunosorbent assay (ELISA) to the determination of pesticides. ELISA's are based on the combination of selective antibodies attached to solid supports with sensitive enzyme reactions. These features produce an analytical system capable of detecting very low levels of chemicals. The immunochemical reaction provides high selectivity due to the extraordinary discriminatory capability of antibodies. The powerful catalytic ability of the enzyme provides highly sensitive detection. Direct sample analysis reduces the dependence on organic solvent extractions.

The specificity of each RaPID Assay method is described in terms of its antibody cross-reactivity to other related compounds. The cross-reactivity of each RaPID Assay with various analogues can be expressed as the least detectable dose (LDD) estimated at 90% B/Bo. A cross-reactivity of NR means there is no reactivity with that compound up to 10,000 ppb.

The well-to-well and tube-to-tube variation commonly associated with coated tube or coated plate assays is eliminated by the use of antibodies covalently attached to magnetic particles.

Alachlor

Rapid – results in less than 60 minutes.
Precision – within assay and between assay %CV < 6% at 0.5 ppb.
Sensitivity – LDD of 0.05 ppb.
Range – 0.05 to 5.0 ppb Alachlor.
Cross-Reactivity – chloroacetanilide analogues and other pesticides.

Compound	LDD (ppb)
Alachlor ethane sulfonic acid (ESA)	0.03
Alachlor	0.05
Butachlor	5.6
Metolachlor	6.0
Propachlor	6,000.

Aldicarb

Rapid – results in less than 60 minutes.
Precision – within assay %CV 17% and between assay %CV < 10% at 12 ppb.
Sensitivity – LDD of 0.25 ppb.
Range – 0.25 to 100 ppb Aldicarb.
Cross-Reactivity – aldicarb analogues and other pesticides.

Compound	LDD (ppb)
Aldicarb	0.25
Aldicarb sulfone	0.27
Aldicarb sulfoxide	1.80
Methomyl	10.0
Acetylcholine	NR
Phosphamidon	NR

Atrazine

Rapid – results in less than 45 minutes.
Precision – within assay %CV < 5% and between assay %CV < 3% at 2 ppb.
Sensitivity – LDD of 0.046 ppb.
Range – 0.046 to 5.0 ppb Atrazine.
Cross-Reactivity – triazine analogues and other pesticides.

Compound	LDD (ppb)
Propazine	0.033
Atrazine	0.046
Ametryn	0.053
Prometryn	0.054
Prometon	0.056
Desethyl Atrazine	0.062
Terbutryn	0.090
Terbutylazine	0.310
Simazine	0.340
Desisopropyl Atrazine	0.800
Cyanazine	1.00
6-Hydroxy Atrazine	1.10

Benomyl/Carbendazim

Rapid – results in less than 50 minutes.
Precision – within assay %CV < 6% and between assay %CV < 4% at 3 ppb.
Sensitivity – LDD of 0.1 ppb Carbendazim.
Range – 0.1 to 5.0 ppb Carbendazim.
Cross-Reactivity – benzimidazole fungicides and other pesticides.

Compound	LDD (ppb)
Carbendazim	0.10
Benomyl	0.38
2-Benzimidazolylurea	0.62
Thiabendazole	6.3
Thiophanat-methyl	19
2-Aminobenzimidazole	120
Benzimidazole	681

Captan

Rapid - results in less than 80 minutes.
Precision - within and between assay %CV < 10% at 0.6 ppm.
Sensitivity - LDD of 10 ppb.
Range - 0.01 to 3.0 ppm Captan.
Cross-Reactivity - phthalimide fungicides, metabolites and other pesticides

Compound	LDD (ppm)
Captan	0.01
Captafol	1.00
Metolachlor	2.00
Propachlor	5.00
Carbaryl	5.00
Folpet	8.60
THPI	10.0
Alachlor	10.0
Phthalimide	NR

Carbaryl

Rapid - results in less than 50 minutes.
Precision - within assay %CV 8% and between assay %CV 5% at 2 ppb
Sensitivity - LDD of 0.25 ppb.
Range - 0.25 to 5.0 ppb Carbaryl.
Cross-Reactivity - carbamate fungicides, metabolites and other pesticides

Compound	LDD (ppb)
Carbaryl	0.25
Carbofuran	7.00
Pentachlorophenol	8.00
Iprodione	12.0
Dichlorophenol	25.0
1-Naphthol	495
Aldicarb	NR
Methomyl	NR

Carbofuran

Rapid - results in less than 50 minutes.
Precision - within assay %CV < 9%, and between assay %CV ≤ 3%, at 2 ppb.
Sensitivity - LDD of 0.056 ppb.
Range - 0.06 to 5.0 ppb Carbofuran.
Cross-Reactivity - carbamates fungicides, metabolites and other pesticides.

Compound	LDD (ppb)
Carbofuran	0.056
3-Keto Carbofuran	1.2
3-Hydroxy Carbofuran	16
EPTC	340
3-Keto Carbofuran phenol	380
Carbaryl	740
3-Hydroxy Carbofuran phenol	1,700
Carbofuran phenol	3,000

2,4-D

Rapid - results in less than 60 minutes.
Precision - within assay %CV < 8% and between assay %CV < 12% at 36 ppb.
Sensitivity - LDD of 0.7 ppb.
Range - 0.7 to 50 ppb 2,4-D.
Cross-Reactivity - chlorophenoxy analogues and other pesticides.

Compound	LDD (ppb)
2,4-D Propylene Glycol Ester	0.05
2,4-D Ethyl Ester	0.05
2,4-D Isopropyl Ester	0.07
2,4-D Methyl Ester	0.12
2,4-D Sec-Butyl Ester	0.13
2,4-D	0.70
2,4,5-T	2.98
2,4-DB	3.95
MCPA	7.80
4-Chlorophenoxy-acetic acid	61
Silvex (2,4,5-TP)	167
Triclopyr	830

Cyanazine

Rapid - results in less than 45 minutes.
Precision - within assay %CV < 10% and between assay %CV ≤ 1% at 0.5 ppb.
Sensitivity - LDD of 0.035 ppb.
Range - 0.04 to 3.0 ppb Cyanazine.
Cross-Reactivity - triazine analogues and other pesticides.

Compound	LDD (ppb)
Cyanazine	0.035
Terbutylazine	0.050
Terbutryn	0.110
Ametryn	0.580
Prometryn	1.5
Simazine	1.6
Propazine	3.5
Prometon	82
Desethylatrazine	117
Atrazine	200

Metolachlor

Rapid - results in less than 60 minutes.
Precision - within assay %CV and between assay %CV < 6% at 0.7 ppb
Sensitivity - LDD of 0.05 ppb.
Range - 0.05 to 5.0 ppb Metolachlor.
Cross-Reactivity - chloroacetanilide analogues and other pesticides.

Compound	LDD (ppb)
Metolachlor	0.05
Acetochlor	0.06
Metalaxyl	0.06
Butachlor	0.26
Propachlor	1.0
Alachlor	1.3

Chlorothalonil

Rapid - results in less than 60 minutes
Precision - within and between assay %CV < 5% at 1.5 ppb
Sensitivity - LDD of 0.07 ppb
Range - 0.07 to 5.0 ppb Chlorothalonil
Cross-Reactivity - phthalimide fungicides and other pesticides

Compound	LDD (ppb)
Chlorothalonil	0.07
Pentachloronitrobenzene	0.14
Hexachlorobenzene	0.16
2,4,5,6-Tetrachloro-3-cyanobenzamide	0.29
2,5,6-Trichloro-4-hydroxyisophthalonitrile	18.7
Pentachlorophenol	29.2
3-Carbamyl-2,4,5-trichloro-benzoic acid	48.1

Pentachlorophenol

Rapid - results in less than 60 minutes
Precision - within assay %CV < 8% and between assay %CV 2% at 3 ppb
Sensitivity - LDD of 0.06 ppb
Range - 0.06 to 10.0 ppb Pentachlorophenol
Cross-Reactivity - organochlorine pesticides and other compounds

Compound	LDD (ppb)
Pentachlorophenol	0.06
2,3,5,6-Tetrachlorophenol	0.21
2,3,4,6-Tetrachlorophenol	0.91
2,3,5-Trichlorophenol	1.52
2,4,6-Trichlorophenol	2.44
Tetrachlorohydroquinone	8.70
2,4,5-Trichlorophenol	15.[illegible]
2,3,5-Trichlorophenol	21.[illegible]
2,3-Dichlorophenol	62.9
2,6-Dichlorophenol	266
Hexachlorobenzene	1,560
3,4-Dichlorophenol	1,670
Hexachlorocyclohexane	5,790
Creosote	NR
DA	NR

Procymidone

Rapid - results in less than 60 minutes
Precision - within assay %CV < 5% and between assay %CV < 6% at 20 ppb
Sensitivity - LDD of 0.8 ppb
Range - 0.80 to 100 ppb Procymidone
Cross-Reactivity - dicarboximide analogues and other pesticides

Compound	LDD (ppb)
Procymidone	0.80
Vinclozolin	170
Iprodione	NR
Carbendazim	NR
3,5-Dichloroaniline	NR

MICROBIOLOGICAL CHARACTERIZATION OF THE WATER AND SEDIMENT IN KAPAHULU STORM DRAIN SYSTEM AND AT KUHIO BEACH

Kimberly K. Roll and Roger S. Fujioka

Project Completion Report KSDS-4

March 1994

for

The Department of Health
State of Hawaii
P. O. Box 3378
Honolulu, HI 96801

Contract No. ASO Log No. 92-613

Project Period: April 1, 1992 to December 31, 1994

Principal Investigator: Roger S. Fujioka
Water Resources Research Center
University of Hawaii
2540 Dole Street
Honolulu, Hawaii 96822

I. MOTIVATION FOR STUDY

A. Use of Bacterial Indicators to Assess Water Quality

Sewage is the source of many microbial pathogens which are transmitted to man by ingestion. When recreational waters are contaminated with sewage, the water can serve as the vector for the transmission of diseases to man and therefore these sewage-borne pathogens are also called water-borne pathogens. The number and types of sewage-borne pathogens (protozoa, bacteria, viruses) are numerous. Moreover, different methods must be employed to recover the different pathogens and for some pathogens, recovery methods are not yet available. Thus, analyzing recreational waters for the presence of all the possible pathogens is time consuming, expensive and simply not feasible. A feasible way to determine the hygienic quality of recreational waters is to determine the concentration of a group of bacteria which is naturally found in the feces of man and warm blooded animals. These groups of bacteria are called indicators of fecal contamination. The criteria (Dutka, 1973, Sloat and Zeil, 1987) for selecting a good indicator of fecal contamination are:

(1) It must be consistently and exclusively associated with the source (feces, sewage) of the pathogens.

(2) It must occur in much greater numbers than the pathogens.

(3) It must not be able to proliferate in the environment.

(4) It must be as stable under environmental conditions and to disinfectants as pathogens.

(5) There must be a simple and unambiguous test for the enumeration of the indicator organism.

B. Old and New EPA Recreational Water Quality Standards

Coliform bacteria, characterized as gram-negative, non-spore forming rods that ferment lactose to form gas within 48 hours at 37 °C was the first group of bacteria used as indicator of fecal contamination of recreational waters. Initially, the proposed guideline for acceptable swimming water in the U.S. was not to exceed 1000-2000 colony forming units (CFU)/100 ml of total coliform bacteria (Scott, 1932). This guideline was based on the levels of coliforms in Connecticut's shoreline water at the time, with 92% of the beaches passing the grade. And, conveniently, little intervention was needed in meeting these standards (Dufour, 1984). In the mid-1960s, it was determined that fecal coliform, a thermotolerant subgroup of total coliform, was a better indicator of fecal contamination. From 1968 to 1986, the recreational water quality standard used in the United States was set at 200 fecal coliforms CFU/100 ml (U.S. EPA, 1976).

In the mid-1970s the Environmental Protection Agency (EPA) conducted an extensive microbiological water quality and epidemiological study, using improved design to determine the predictability of bacterial indicator concentrations in recreational waters and incidences of gastroenteritis diseases among swimmers. The results of that study showed that concentrations of total coliform and fecal coliforms were unreliable predictors for water-borne diseases. However, concentrations of only enterococci bacteria in marine waters and enterococci as well as Escherichia coli in fresh waters were reliable predictors for the incidences of gastroenteritis diseases among swimmers (Cabelli, 1983). Due to the findings of the Cabelli study, the EPA in 1984 initially established 3 enterococci CFU/100ml of sample as the standard for marine water quality and 20 enterococci CFU/100ml or 77 E. coli CFU/100ml for fresh water (U.S. EPA, 1984). These standards were changed in 1986 to make the criteria more closely approximate the historically accepted level of 200 fecal coliform. Thus in 1986 EPA revised their recommendations to 35 enterococci CFU/100ml for marine water and 33 enterococci CFU/100ml or 126 E. coli CFU/100ml for fresh water (U.S. EPA, 1986).

C. Appropriate standards for Hawaii and Tropical Islands

In Hawaii, Guam, and Puerto Rico, high concentrations of fecal indicators have been recovered from environmental waters with no known sources of sewage contamination (Fujioka et al., 1988, Hardina and Fujioka, 1991, Hazen, 1988). For example, Hardina and Fujioka (1991) recovered average levels of 7,813 E. coli CFU/100ml and 3,220 enterococci CFU/100ml in Manoa stream water, far exceeding the Federal standard for fresh recreational water of 126 CFU/100ml of E. coli and 33 CFU/100ml for enterococci . Moreover, these fecal indicator bacteria were present in all soils at high concentrations ranging from 100 to 10,000 per/100 g of soil. It was therefore, hypothesized that these indicator bacteria were multiplying in the soil and were being washed into the streams by rain.

If these fecal indicator are naturally present in Hawaii's soil and moreover are multiplying in the soils of Hawaii, the following two assumptions and criteria for a good fecal indicator are not valid in Hawaii: 1. The indicator must be consistently and exclusively associated with feces, 2. The indicator must not multiply outside of the human intestinal tract. These results indicate that the recreational water quality standards which are recommended by USEPA are not applicable to Hawaii and other tropical islands.

To find a reliable indicator of recreational water quality in Hawaii, Fujioka and Shizumura (1985) tested alternative indicators of water quality and determined that Clostridium perfringens was superior to the indicators recommended by USEPA in Hawaii. C. perfringens is an anaerobic spore-forming bacilli which is naturally found in the intestinal tract of humans and warm blooded animals. Since C. perfringens is an anaerobe, it cannot multiply in the environment. Moreover, it is the spores of C. perfringens which persist in the environment. Since spores are extremely resistant, C. perfringens is considered too stable and is considered to be a too conservative indicator by USEPA.

However, in tests conducted in Hawaii, C. perfringens was found in relatively low numbers as compared to fecal coliforms in streams not receiving sewage effluent, while in

stream samples taken below sewage discharge sites, consistently high levels of C. perfringens were found. In assessing the use of C. perfringens, Fujioka and Shizimura, (1985) concluded that it was the best indicator for the presence or absence of sewage and recommended that for stream water, a standard of not more than 50 C. perfringens/100 ml be used. Use of C. perfringens was extended to coastal marine waters where it was recommended that concentrations of C. perfringens should not exceed 5 CFU/100 ml and deep coastal waters should not exceed 2 CFU/100 ml of this bacteria.

To better determine the quality of coastal waters Fujioka (1990) further determined that the aerobic spore forming bacteria (bacillus spores) which are naturally found in the soil could be used as an indicator that recreational waters are contaminated with soil. Since soil is the source of fecal coliform, enterococcus, E. coli and bacillus spores, coastal waters which contain all of these bacteria can be taken as evidence that the source of these bacteria is soil. On the other hand, coastal water samples which contain C. perfringens, as well as enterococci or E. coli in the absence of bacillus spores may be taken as evidence that the source of contamination is sewage.

D. Water Quality of Urban runoff and Storm Drains

Storm water runoff has for many years been considered as nonpoint sources of pollution and therefore the quality of this type of water has generally not been monitored. However, several studies have shown that storm drain waters can contain high levels of fecal indicator bacteria and occasionally pathogens as well (Oliveri et al. 1977). Average densities of 2.38×10^3 fecal coliform CFU/100 ml were recovered by Davis (1979) in a study of urban and rural storm water runoff quality in Texas.

Several studies have been conducted on the bacterial water quality of storm drains in Hawaii. In 1972, Chun et al recovered fecal coliform levels as high as 700/g from dirt and dust from three Honolulu streets. Rain wash these dirt and soil into storm drains. In 1978, Young, reported a range of 463 - 2.0×10^3 fecal coliform/100ml and 6.3×10^3 - 7.9×10^3 fecal streptococcus/100ml (MPN) in Honolulu's storm drains. In a more recent study, Fujioka (1988) recovered a range of 4.7×10^3 - 1.8×10^5 fecal coliform CFU/100ml and 1.2×10^3 - 1.1×10^5 fecal streptococcus CFU/100ml in rural and urban storm drains. In 1988, Fujioka reported that fecal indicator bacteria were recovered from streams in pristine areas of the mountains and generally increase as the stream water pass through urbanized areas where storm drains discharge into streams.

In 1990, Fujioka documented that high concentrations of fecal indicator bacteria in storm drains and streams were adversely affecting the quality of coastal waters which receive these waters. In that report, Fujioka concluded that fecal indicator recovered from storm drains and streams of Hawaii which do not receive sewage effluents should have less of a public health significance as compared to fecal indicator recovered from sewage. This same issue was addressed by Cabelli who was responsible for designing the EPA studies which led to the presently accepted water quality standards using enterococci and E. coli. Cabelli (1989) reported that the water quality standards are only effective for waters polluted by municipal

wastewater and sewage sludge discharges. The standard is not applicable when bodies of water are contaminated with fecal indicator bacteria whose sources are bathers themselves, sanitary wastes from boats, storm water runoff and direct discharges from lower animals.

II. IDENTIFYING A PROBLEM IN HAWAII

A. New Recreational Water Quality Standards for Hawaii

In 1986, USEPA recommended that every state change the marine recreational water quality criteria and standards from a geometric mean of 200 CFU/100ml of fecal coliform to a geometric mean of 35 enterococci CFU/100ml. The federal standard of 35 enterococci CFU/100ml correlated with 19 cases per 1000 swimmers. This was unacceptably high for the State of Hawaii. As a result, the state of Hawaii in 1990 established its marine recreational water quality standard at 7 enterococci/100 ml based on the EPA prediction that at a geometric mean of 7 enterococci/100 ml, the predictable and acceptable disease rate was 10 diseases per 1000 swimmers (DOH, State of Hawaii, 1990). Of all the states in the U.S., this is the most restrictive recreational water quality standard. However, the state of Hawaii has still retained the old standard of 200 fecal coliform CFU/100 ml for inland waters designated for recreational use because of evidence of naturally high concentrations of indicator bacteria in Hawaii's streams.

B. Review of beach water quality by State DOH

In 1991, the Hawaii State Department of Health reviewed the historical enterococcus and fecal coliform data (1973 - 1990) from nine sites along Waikiki beach, spanning the coastline from Ala Moana Bridge to the Elk's Club Beach (Harrigan, 1991). A decreasing trend in the levels of the indicator bacteria was observed in a southeasterly direction away from the Ala Wai Canal and toward the Diamond Head end of Waikiki. However, one exception to this trend was near Kuhio Beach, a popular swimming area enclosed by a low seawall and adjacent to where the Kapahulu Storm Drain System empties into the ocean. Over the 5-year period that enterococci data from this area were reviewed, 46 % of the samples exceeded the Hawaii State Marine Recreational Standard of 7 enterococci CFU/100ml. In 1990 Kuhio Beach was found to "chronically" exceed the standard with 71% of the geometric means calculated above the standard. The term chronic refers to situations when 50% of the geometric means calculated in a calendar year exceed the state standard.

As a result of the elevated concentrations of enterococci at Kuhio Beach, the DOH was obliged to determine the source of enterococci, the risk to humans associated with the elevated counts of enterococci in the water at Kuhio Beach and to make recommendations for management actions if necessary. However, in reviewing the conditions at Kuhio Beach, it was clear that the most obvious source of indicator bacteria was not sewage, which is a definite source of water borne diseases but was the Kapahulu storm drain which has an outlet under the jetty bordering the southeastern end of Kuhio Beach. In studies conducted earlier by Water Resources Research Center of the University of Hawaii, it was clearly demonstrated

that storm drains in Hawaii contain very high concentrations of fecal indicator bacteria and especially enterococci (Fujioka et al., 1988). Moreover, beaches that receive discharge of stream or storm drains can expect to have elevated concentrations of indicator bacteria, occasionally exceeding the marine recreational water quality standard (Fujioka 1990). Thus, the most obvious source of enterococci recovered from Kuhio Beach is the storm drain water for Kapahulu storm drain system. However, data to clearly document that the Kapahulu storm drain is the source of the enterococci in Kuhio Beach is not available.

Clearly, the most important question is whether the source of enterococci bacteria in the waters at Kuhio Beach signals a health risk to people using that water. The USEPA study upon which the enterococci marine recreational water quality standard is based provided evidence that at marine beaches with known sources of sewage contamination, elevated concentrations of enterococci in recreational waters will result in an increase in gastrointestinal illness among swimmers in that water (Cabelli, 1983). In a follow up study by the USEPA, it was shown that if the source of indicator bacteria (E. coli, enterococci) polluting a body of recreational water is not sewage but a non-point source presumably of animal waste, the elevated concentrations of indicator bacteria in recreational water was not a reliable predicator of illness among swimmers (Calderon et al., 1991).

In summary, it was documented that waters at Kuhio Beach contain concentrations of enterococci often exceeding the new recreational water quality standard of 7 enterococci CFU/100ml. Although there is no direct data, the available information strongly indicate that the major source of enterococci bacteria at Kuhio Beach comes from the discharge of storm drain water from the Kapahulu storm drain. Although, elevated concentrations of enterococci in recreational waters is interpreted by the USEPA to be associated with increase in illness among swimmers the conditions at Kuhio Beach present circumstantial evidence that this may not be the case. However, additional evidence is required to strengthen the interpretation of water quality at Kuhio Beach. In this regard, two study approaches are required to provide additional information. First, there is a need to monitor the levels of indicator bacteria in the Kapahulu Storm Drain System and determine if this is a source of indicator bacteria recovered from Kuhio Beach. Second, there is a need to conduct a water quality and epidemiological study similar to that conducted by the USEPA to measure actual disease incidences among the swimmers at Kuhio Beach.

III. OBJECTIVES OF STUDY

A microbiological team was established to complete the following three separate objectives of the multiphasic studies of this project:

1. To conduct a microbiological water quality assessment of the Kapahulu Storm Drain System and to determine the sources of indicator bacteria recovered from the Kapahulu Storm Drain System.

2. To determine the impact of this storm drain on the bacterial quality of water at Kuhio Beach.

3. To provide the water quality data at Kuhio Beach for the epidemiological study which is described in a companion study.

IV. STUDY SITES AND SAMPLING STATIONS

A. Establishing the Study Area and Sampling Sites

Mr. Chew Lun Lau of the Department of Public Works (DPW), City and County of Honolulu (CCH) was initially consulted to obtain blue prints and information about the Kapahulu Storm Drain System (KSDS). A crew from CCH showed members of the microbiological team the sampling stations at the KSDS and how to open the storm drain covers to sample the waters. The cooperation and information from the DPW and the Department of Parks and Recreation, CCH were invaluable to this study.

A sketch of the study area which includes the Kapahulu Storm Drain System (KSDS), Kuhio Beach area, and the locations of the sampling sites are outlined in Figure 1. The figure clearly shows that two major tributaries of the KSDS contribute to the water being discharged into Kuhio Beach. The west branch of the tributary collects water primarily from urbanized area and was designated the "urbanized tributary". The east branch of the tributary collects water primarily from the Waikiki Hotel area and was designated the "hotel tributary". Sampling sites were selected to characterize the water in the KSDS and the water at Kuhio Beach.

B. The Urbanized tributary of Kapahulu Storm Drain System (KSDS)

Site 12. This site collects the water draining from the urbanized community before it enters the Zoo area. Located on Monsarrat Ave at the north end of the Waikiki Shell parking lot, it is an open gully which receives runoff from the Diamond Head area via a channel running under Kapiolani Park. Under dry conditions water trickles through the pipe under Monsarrat Ave. toward the Zoo area. The area surrounding site 12 is overgrown with weeds and became more so over the course of the study.

Site 11. This site is approximately 100 meters downstream of site 12 and is located approximately mid-point of the Honolulu Zoo between the turtle and monkey displays. This site was selected because there was some concern that waste waters from the Waikiki Zoo were entering the KSDS. Water from this site represents storm drain water from the urbanized area and the contributions of storm drain water from the Zoo area.

Site 10. This site is approximately 200 meters downstream from site 11 and is located in the Zoo parking. Water from this site contains the storm drain water from Site 11 and also from storm water draining the Kapahulu Avenue area.

C. The Hotel Tributary of KSDS

Site 8. This site collects the water draining from a Waikiki hotel area and is located on the east sidewalk of Ohua St., approximately 75 meters up from Kalakaua Ave.

Site 9. This site is approximately 150 meters downstream from site 8 and is at the intersection of Paoakalani Street and Kalakaua Avenue. It is a low spot and therefore is under tidal influence. Thus, water from this site represents water from the hotel tributary area mixed with some intruding ocean water.

D. Ocean Sites Near the Discharge of KSDS

Site 5. This site is at the mouth of the discharge site for the KSDS into the Kuhio Beach area and is located at the end of the stone jetty. Samples of water were taken at the large opening at the end of the jetty. Water from this site represents the initial dilution of the storm drain water from the KSDS with the ocean water.

Site 4. This site is located approximately 75 meters west of Site 5 and is seaside of the seawall between Sites 1 and 2. This site is susceptible to contamination from Site 5 but the water exchange rate at this site is good.

Site 6. This site is located just east of Site 5 and can be expected to receive some of the water from Site 5. Water circulation at this site is good.

E. Sites Within the Enclosed, Swimming Area of Kuhio Beach

Site 1. This site represents the eastern half of the enclosed portion of Kuhio Beach and is used extensively for swimming. There is an opening of the seawall near Site 5 which allows water from Site 5 to enter this enclosed area.

Site 2. This site represents the western portion of the enclosed area of Kuhio Beach and is a popular swimming area. This site is approximately 100 meters from Site 5 and the sea wall near this site has a 20 meter opening to increase water circulation.

F. Control, Unimpacted Sites

Site 3. This site is located approximately 200 meters northwest from Site 5 and is west of Site 2. This area is outside of the seawall enclosing Kuhio Beach and therefore has good water circulation. Thus, this site is not expected to be measurably impacted by storm drain water from Site 5 and this site was selected as a control, non-storm drain impacted site.

Site 7. This site is near Queen's Surf Beach which is approximately 200 meters southeast of Site 5. There is good water circulation at this site and no discharge of storm water near this site. Thus, this site was selected as a control, non-storm drain impacted site.

V. METHODOLOGY

A. Microbiological Analysis of Water Samples

For microbiological analysis, surface water was collected in sterile polyethylene bottles. Samples were stored in an iced cooler and transported to the laboratory to be analyzed within 6 hrs.

The enumeration of bacteria in the ocean and storm drain water samples was performed using the membrane filtration technique as outlined in Standard Methods of Water and Wastewater 17th ed. (APHA, 1989). For recovery of enterococcus bacteria the membrane was initially placed on Difco mE agar and incubated for 48 hr at 41 °C. The membranes were then transferred to Difco Esculin Iron Agar (EIA) plate and incubated at 41 °C for 20 min. Positive enterococci colonies were pink to red colonies that developed a black of reddish-brown precipitate on the under side of the filter. For recovery of fecal coliforms, membranes were placed on Difco mFC media and incubated for 24 hr at 44.5 °C in a water bath. Positive fecal coliform colonies were blue colonies. For recovery of E. coli, membranes were placed on Difco mTEC media, and incubated for 2 hr at 30 °C as a resuscitation step, followed by a 22 hr incubation in a water bath at 44.5 °C. The membrane was then transferred to a filter pad saturated with urease. After 15 min. positive E. coli colonies were yellow or yellow-brown colonies. For recovery of C. perfringens, the membranes were placed on mCP media (Bisson and Cabelli, 1979) and incubated for 24 hr under anaerobic conditions at 45 °C. Positive C. perfringens colonies turn from a yellow color to pink on exposure to ammonium hydroxide. For the recovery of bacillus spores, 200 ml of water samples were initially pasteurized in water bath at 63 °C for 1 h to inactivate vegetative cells but to allow bacterial spores to survive. The membrane was then placed onto mTGA media and incubated at 37 °C for 48 hr. Positive bacillus spores developed into black colonies.

B. Microbiological Assay of Soil

Sterile 500 ml plastic containers were filled approximately half way with soil samples which were kept on ice and transported to the lab and analyzed with in 4 hr.

The Most Probable Numeration (MPN) method (APHA, 1989) was used to enumerate fecal coliforms, C. perfringens and fecal streptococcus in the soil samples. 10 g of samples were added to 99 ml of sterile phosphate buffer solution to obtain a concentration of 0.1 g/ml. A series of concentrations (.01, .001, and .0001) were then made by transferring 10 ml of the higher concentration to 90 ml of sterile phosphate buffer solution until the lowest concentration was obtained. In order to obtain the MPN/ Index/g for the bacteria, the following equation was used for calculation:

MPN Index / 100 ml = MPN Index x 10 / largest volume tested (1 ml = 0.1 g, thus 100 ml = 10 g)

Therefore: MPN Index / 100ml = MPN Index / 10 g, and dividing the MPN Index by 10 will give an MPN Index/g for the sample.

EC+MUG medium was used in the presumptive phase for fecal coliform. Tubes were incubated for 24-48 hr. at 44.5 °C. All positive tubes (growth and acid) within 48 hr were confirmed (growth and acid) using EC broth and incubated at 44.5 °C. The MUG fluorescence test was also used to confirm the presence of E. coli in all positive presumptive tubes. Sulfite-Polymyxin-Sulfadiazine (SPS) broth was used in the presumptive phase for C. perfringens. Tubes were anaerobically incubated at 37 °C for 24 hr. Positive tubes (turbidity or growth) were streaked on mCP agar (Bisson and Cabelli, 1979) in the confirmed phase and incubated at 41 °C for 24 hr. Red to burgundy colonies upon exposure to ammonium hydroxide vapors were confirmed as C. perfringens. Azide-dextrose broth was used in the presumptive phase for fecal streptococci. All tubes were incubated at 35 °C for 24-48 hr. Positive tubes (turbidity or growth) were streaked on PSE agar for confirmation and incubated at 35 °C for 24 hr.

To enumerate indicator bacteria from sand samples the method previously reported by Oshiro (1990) was used. Elutions of the sand were made by adding 90 ml of sterile phosphate buffer to 90 g of sand sample, which was shaken vigorously for 10 seconds and let sit for a minute before the supernatant was poured into a sterile container. Another 90 ml of sterile buffer was added to the same sand sample and again shaken and the supernatant poured into the same sterile container. The eluate was then analyzed for levels of enterococci, E. coli, fecal coliform, C. perfringens and bacillus spores using the methods to enumerate bacteria in the ocean and storm drain water samples described above.

C. Measurement of Physical Parameters in Water Samples

Acid washed borosilicate bottles were used to collect water samples for reactive phosphorus and pH analysis. Reactive phosphate mg/L (PO_4) was measured within 24 hr using the HACH method and the HACH DR 3000 Spectrophotometer. The values were converted to mg/L reactive phosphorus by dividing by three. pH was measured (within 6 hours of collection) using the Orion pH meter (Model 811).

Dissolved oxygen was measured at the site using a Yellow Springs Instrument Co. Model 57 Dissolved Oxygen Meter. Salinity was also measured on site using a refractometer.

D. Measurement of Dye As Tracer

The organic fluorescent dye tracer, fluorescein, which is non-toxic at low levels was selected because it has a low sensitivity to both salinity and temperature changes, and does not

adsorb to walls. Although the fluorescence of this dye is reduced in a low pH environment and has a high photo decay rate (Smart and Laidlow, 1977), these conditions are minimized when the tracer is used in a storm drain.

The storm drain and sewer line samples were collected in 15 ml borosilicate glass containers and kept in the shade. The samples were processed within 6 hours. The levels of fluorescein in the water samples were determined visually and measured using a Turner Filter fluorometer (Model 111). The fluorometer measures the relative intensity of light emitted from a liquid containing fluorescent material and the amount of fluorescein in proportion to the amount of fluorescent material in the liquid. Calibration curves were constructed for each sample, with known amounts of fluorescein. Because of the high background fluorescence levels in storm drain waters, calibration curves were constructed for each sample, by measuring the sample water and by adding various known amounts of fluorescein dye to sample water as compared to distilled water.

VI. RESULTS: QUALITY OF WATER AND SEDIMENT IN KSDS

The quality of the water and sediment in the Kapahulu Storm Drain System (KSDS) was determined by obtaining and analyzing 18 water and sediment samples from five carefully selected sites (see Figure 1). Samples were analyzed for indicator bacteria (enterococci, E. coli and fecal coliforms, C. perfringens, bacillus spores) and some chemical parameters (pH, salinity, dissolved oxygen, reactive phosphorus). The results of these analyses are summarized in Table 1, Figure 2 and detailed in Appendices 1 - 5.

A. Storm Drain from Hotel Tributary of KSDS

1. Site 8. Water samples from Site 8 represent storm drain water produced from hotels and other retail outlets which are concentrated in the Waikiki area. Water at this site appeared stagnant and characterized by low salinity (Average: 0.9 ppt), indicating that water at this site was essentially fresh and was not being mixed with ocean water or groundwater. Of all the storm drain sites tested, water at this site contained the highest reactive phosphorus level (Average:1.26 mg/l) indicating that run off from this hotel site contain elevated concentrations of nutrients. Sources of phosphorus from hotel areas which can be expected to enter storm drains are food products, cleaning solutions, and fertilizers.

Geometric mean concentrations of fecal indicator bacteria (enterococci: 890 CFU/100 ml, E. coli: 9,291 CFU/100 ml, fecal coliform: 24,081 CFU/100 ml) at Site 8 greatly exceeded the recreational water quality standards based on these indicator bacteria. However, the low concentrations of C. perfringens (11 CFU/100 ml) indicate that the source of these indicator is environmental rather than from sewage or feces of animals. The concentrations of bacillus spores (24 CFU/100 ml) was moderate and indicative of contribution of soil. In assessing the concentrations of indicator bacteria from site 8, it should be noted that water at this site was not diluted by brackish water.

At Site 8, the water samples often had a turbid appearance and a foul odor. Moreover, there was a period when the water had an opaque milky color appearance and this water foamed when mixed. On July 2, 1993, the City and County was called to investigate the discolored storm drain water. As a result of this investigation, an illegal hook up to the storm drain system was discovered. A sump in the basement of a near-by hotel was found to lead directly into the storm drain line and was found to contain the same milky white substance. The illegal connection was sealed and since that time water from site 8 has not appeared milky.

2. Site 9. This site is just downstream of Site 8 and just before the storm drain water from the hotel area merges with the transport and discharge of all storm from the KDSD into the ocean water near Kuhio Beach. Water samples from Site 9 were characterized by elevated salinity (Average: 13.8 ppt) indicating that storm drain water at this site is brackish. Since Site 9 is at a low elevation, some ocean water can be expected to intrude to this site. Water from this site contained acceptable levels of dissolved oxygen (Average: 5.4 mg/l) and pH (7.73) and slightly elevated concentrations of reactive phosphorus (Average 0.331 mg/l).

Geometric mean concentrations of all fecal indicator bacteria (enterococci: 241 CFU/100 ml, E. coli: 671 CFU/100 ml, fecal coliform: 1,492 CFU/100 ml) were lower at Site 9 than at Site 8 but still exceeded the recreational water quality standards based on these indicator bacteria. The low geometric mean concentration of C. perfringens (5 CFU/100 ml) in the same water samples indicates that the source of these fecal indicator bacteria were environmental rather than from sewage or animal feces. Elevated geometric mean concentrations of bacillus spores (70 CFU/100ml) in water samples from Site 9 may reflect the accumulation of dirt and debris between Sites 8 and 9. In assessing the lower concentrations of all fecal indicator bacteria at Site 9 as compared to Site 8, it should be noted that water from Site 9 has been diluted with ocean water and is brackish. Moreover, it is well known that inactivation of indicator bacteria occurs faster in brackish water than in fresh water.

B. Storm Drain from Urbanized Tributary of KSDS

1. Site 12. Water from Site 12 represents storm drain water produced by an urbanized community. Water flow from Site 12 was usually a slight trickle as it flowed under Monsarrat Ave and the water usually had a brown and turbid appearance. Water samples from this site had an average salinity of 11.8 ppt indicating that the storm drain water from this urbanized area was also brackish. The relative elevation of Site 12 was still low enough to allow ocean water at high tides to intrude into this area. Water samples had acceptable levels of pH (Average: 7.78) and low dissolved oxygen (Average: 2.2 mg/l). The average concentrations of 0.757 mg/l of reactive phosphorus in the water at this site was elevated indicating that fertilizers or perhaps organic debris were being added to this storm drain system.

Geometric mean concentrations of fecal indicator bacteria (enterococci: 3,975 CFU/100 ml, E. coli: 6,270 CFU/100 ml, fecal coliform: 5,961 CFU/100 ml in water samples from Site 12 greatly exceeded the recreational water quality standards based on the

concentrations of these bacteria. The elevated concentrations of C. perfringens (147 CFU/100 ml) in these same water samples indicate that sewage or feces from animals is a major contribution to the storm water at Site 12. Urbanized communities are characterized by pets whose feces often are washed into the storm drain. Elevated concentrations of C. perfringens in storm drains as compared to free flowing streams in Hawaii were previously reported by Fujioka (1990). Thus, animal pet feces is the most likely source of the elevated concentrations of C. perfringens. The low concentration of bacillus spores (4 CFU/100 ml) indicated less contribution of soil.

2. Site 11. This site is downstream of Site 12 and within the Honolulu Zoo. Thus, run off from parts of the zoo also discharge into this drainage area. More water was observed at this site than at Site 12 indicating that the water values observed at Site 12 may be diluted before it reaches Site 11. Water from Site 11 was characterized as being brackish (average salinity: 15.2 ppt) with acceptable average values for pH (7.81) and dissolved oxygen (3.3 mg/l). The average concentrations of reactive phosphorus (0.188 mg/l) was relatively low and lower than at Site 12.

Geometric mean concentrations of fecal indicator bacteria (enterococci: 376 CFU/100 ml, E. coli: 572 CFU/100 ml, fecal coliform: 851 CFU/100 ml, in water samples from Site 11 were much lower than in water samples obtained from Site 12 but still exceeded the recreational water quality standards based on these indicator bacteria. The observation of lower concentrations of fecal indicator at Site 11 as compared to Site 12 is probably due to the greater volume of water flowing at Site 11 which reflect a greater dilution. If the zoo is the source of water responsible for this dilution at Site 11, it is evidence that storm drain water from the zoo is not a major source of fecal indicator bacteria. The slightly lower concentrations of C. perfringens (58 CFU/100 ml) at Site 11 as compared to Site 12 may also reflect the dilution effect at Site 11.

3. Site 10. This site is downstream of Site 11 and located in the Zoo parking lot. Thus, the water in the storm drain at this site represents the storm drain water flowing from Site 11 but is also mixed with storm drain water draining the Kapahulu Avenue area as well. The average values for salinity (16.1 ppt), the pH (7.77), the dissolved oxygen (3.5 mg/l) and reactive phosphorus (0.174 mg/l) in the water samples obtained from Site 10 were very similar to that at Site 11. Both Sites 11 and 12 are low elevations which will allow ocean water to intrude to these sites under high tide conditions.

The geometric mean concentrations of the fecal indicator bacteria (enterococci: 1,510 CFU/100 ml, E. coli: 2,089 CFU/100 ml, fecal coliform: 2,145 CFU/100 ml) were much higher at Site 10 than at Site 11 and greatly exceeded the recreational water quality standards based on the concentrations of these indicator bacteria. The concentrations of C. perfringens (31 CFU/100 ml) and bacillus spores (33 CFU/100 ml) were of moderate levels. These results probably reflect the contribution of storm water flowing from Site 11 (urbanized area) and mixed with storm drain flowing from the Kapahulu Avenue area (urbanized/commercial zone).

It should be noted that the highest counts of bacteria in the storm drains were recovered on 9/12/92, the day after hurricane Iniki hit the islands. This was an unusual day but reflects a natural event which can result in extraordinarily high concentrations of fecal indicator bacteria in storm drains and detrimental impact on the quality of beach waters throughout the islands. Rain and resulting high wind and waves contribute to the transport of more forms of pollutants into storm drain during these storm events. On this sampling day, sand was actually washed up onto Kalakaua Ave indicating that sea water, with force, had pushed itself inland and had resulted in cleaning out (suspending) much of the sediment in the storm drains.

VII. SOURCES OF INDICATOR BACTERIA IN THE KSDS

A. Animal Feces from the Honolulu Zoo

There has been much speculation that the fecal wastes from the Honolulu Zoo were being discharged into the storm drain and was the source of most of the indicator bacteria found in the KSDS. However, high concentrations of fecal indicator bacteria were recovered in all storm drain sites, even those not impacted by discharge from the Zoo area. These results are taken as evidence that there are sources of fecal indicator bacteria other than from the Honolulu Zoo. In this regard, the reports of environmental sources (stream, soil) of indicator bacteria in Hawaii have been previously reported (Fujioka et al, 1988; Hardina and Fujioka 1991). However, since the Honolulu Zoo is an obvious source of fecal wastes, a study was conducted to determine its contribution to the storm drain.

All experiments at the Zoo was conducted with the cooperation of Mr. Lloyd Shimazu who showed us the sites and answered all questions on operations at the Zoo. It was determined that the Honolulu Zoo discharges the fecal wastes of animal into a dedicated sewer line which transport this source of waste into the sewage line for the City of Honolulu. Thus, the animal waste from the zoo should not be entering the storm drain. To directly test this hypotheses, fluorescein dye was added as a single slug (one minute dose) into the Zoo sewer line at two sites at two separate times and water samples were taken from storm drains in the Zoo grounds which cross the sewer line down stream from the dye injection sites to determine if the dye enters the storm drain. Samples were also taken from the sewer line down stream from the sites where the dye was added and water samples from this site were also taken. These water samples were taken before the addition of the dye and at least every 10-15 minutes after the addition of the dye. All water samples were visually assessed for dye as well as measured for fluorescence to detect low levels of dye. A map of the zoo showing the dye injection sites and the sampling sites in the sewer line and the storm drain lines are shown in Figure 3. It should be noted that measurable background fluorescence was detected in storm drain water (0.0314 to .226 ppb) and in sewage samples (0.662 ppb) even before the fluorescein dye was added. Thus, background levels of natural fluorescence are present in storm water and sewage.

Fluorescein dye was first added into sewer line within the zoo near the gharial pool at 10:05 AM on August 30, 1993. The results of monitoring the storm drain and the sewage

sampling sites are summarized in Table 2 and graphically displayed in Figure 3. In Figure 3, 0 minutes represents 10:00 AM or 5 minutes before the addition of the dye to the first sewer line site within the zoo at 10:05 AM. As shown in Figure 3, it took approximately 17 minutes before the dye was detected at a level almost 1000 times over background at the sewer line test site on Monsarrat Avenue. Most of the dye flowed past the Monsarrat sewer line site between 30 and 60 minutes after the dye was added, reaching concentrations almost 1,000,000 times above background. During this period, the dye in the sewage line was easily visible to the naked eye and dye was not visible at the storm drain monitoring sites. Since the flow in the sewer line at the zoo was low and inconsistent, fresh water was pumped into the line after the addition of the dye to enhance the transport of the sewage and the dye through the sewage line. Using this method, most of the visible dye was no longer present at the sewage site 60 minutes after the addition of the dye at the injection site near the gharials.

A second dose of fluorescein dye was injected into the sewer line behind the Sun bear cage at 11:15 AM (t = 75 min.) and water samples at the storm drain sites and the sewage line site again monitored for fluorescence. The results are summarized in Table 2 and graphically shown in Figure 3. In this experiment, a sharper and smaller peak of dye was observed at the sewage site approximately 13 minutes after the injection of the dye (85 minutes on Figure 3. The sharper peak represents the addition of less dye in a shorter period of time, a decision made after our knowledge from the first dye injection experiment. During these time periods the water at the storm drain 1 (SD1) test site had fluorescein levels ranging from .0000224 ppm to .000113 ppm while fluorescein levels at and storm drain 2 (SD2) ranged from .0000928 ppm to .002098 ppm. At storm drain 2, there was an increase in fluorescence at the 90 minute time period which could be interpreted as increase of fluorescence due to dye or natural background levels of fluorescence. Since the level of fluorescence detected was below the natural background levels of fluorescent dye, it was concluded that this increase was within the range of background levels of fluorescence as shown by the background fluorescence at Storm drain sites 1 and 2 measured on two separate days (see Figure 3).

In summary, the results of these experiments clearly showed that the sewage from the zoo was being transported directly to the City and County of Honolulu's major sewage line. Moreover, that there was not a direct link between the Zoo's sewer line and the storm drain system. However, our study design does not rule out the possibility of a slow or indirect contamination between the sewage line and the storm drain line. The advantage of using fluorescein dye was the ease of visualizing the dye and the sensitivity of the measurements. The disadvantage of this dye is the presence of low levels of natural background fluorescence in sewage and storm drain water. Thus, in future dye studies, selection of dyes should be based after determining the background levels of fluorescence in the test samples.

B. Soil as a Source of Indicator Bacteria in KSDS

In a previous study, Hardina and Fujioka (1991) reported that high levels of indicator bacteria are naturally present in Hawaii's soil and concluded that soil is the primary source of the indicator bacteria recovered from streams. Moreover, that rain becomes the carrier of these indicator bacteria. Run off from soil is a major contributor to storm water. It was thus

hypothesized that soil is a major source of the bacteria found in storm drains. Soil as a source fecal indicator bacteria is exacerbated when pigeons roost in trees above a grassy area or walk on the grounds of the grassy area. Areas fitting this description are available inside and outside the Honolulu Zoo.

For this study soil samples were obtained from the following three sites: Site 1: grassy area within Honolulu Zoo grounds known to be heavily used by pigeons. Site 2: grassy area outside of Zoo entrance and known to be heavily used by pigeons. Site 3: grassy backyard of private residence near Kapiolani Park which was not used by pigeons. The results of analyzing the soil samples are summarized in Table 3 and show first of all that all three soil samples contained ≥16,000 MPN/g of fecal streptococcus. The concentrations of fecal coliform in soil samples from Sites 1 and 3 were similar (1,000-1300 MPN/g of soil) and higher at Site 2 (≥16,000 MPN/g of soil). These results may reflect the fact that Site 2 is protected from sun and drying by the spreading banyan tree. Similar levels of C. perfringens (300-500 MPN/g) were recovered from soil samples from Sites 1 and 2 with lower levels from soil samples from Site 3 (90 MPN/g). These results indicate that all soil samples in Hawaii whether it is used by pigeons or not contain high levels of fecal coliform and fecal streptococci bacteria and much lower levels of C. perfringens. The feces of pigeons have been previously determined to contain high levels of fecal coliform and fecal streptococci but low levels of C. perfringens. Thus, soil in areas used by pigeons can be expected to have high concentrations of indicator bacteria. However, soils not used by pigeons must still be considered sources of indicator bacteria. It therefore, can be expected that when it rains, bacteria from soil everywhere will be washed into the storm drain systems.

VIII. IMPACT OF KSDS ON KUHIO BEACH

Kuhio Beach is characterized by being essentially enclosed by the rock walled jetty to the east and a line of breakers to the south and west. These man-made walls result in an enclosed body of water which is calm and a major reason why this beach has one of the highest density of swimmers. However, this enclosure results in poor water circulation and is one of the reason why the quality of water at this beach has not been able to consistently meet the state of Hawaii marine recreational water quality standard of 7 enterococci/100 ml. More recently, the storm drain water being discharged from the jetty near Kuhio Beach has been suspected as a source of contaminating the water within Kuhio Beach.

To determine the extent at which the storm water from the Kapahulu Storm Drain System (KSDS) was contributing to the fecal indicator bacterial concentrations in the ocean waters near Kuhio Beach, water samples from selected ocean sites (Sites 1, 2, 5 and 7) at Kuhio Beach and Queen's Surf Beach were sampled concurrently with the five storm drain sites 18 times over the course of the study. Water samples from sites 3, 4 and 6 were concurrently sampled with the storm drain sites on 15 days. Location of each of the sites are shown in Figure 1 and each site is described in detailed in the Methodology Section. The

results of analyzing each water sample for the various bacteria and chemical parameters are summarized in Table 4 and detailed in Tables 5-13.

A. Control Sites. To assess the impact of the discharge of storm drain water near Kuhio Beach, there is a need to determine the quality of ocean water sites which are relatively near, used for the same purpose but not likely to be impacted by the storm drain discharge for comparative purposes. Two of these control sites were selected. The first control site is Site 3 which is located farthest west of Kuhio Beach and just outside the breakers which demarcate the boundaries of Kuhio Beach. There is good ocean current circulation at this site and many people use this area for swimming. The second control site is Site 7 (Queen's Surf Beach) which is located farthest east from Kuhio Beach and is also a popular swimming site. Due to ocean currents and its location, this site was considered least likely to be impacted by storm drain run off into the ocean.

The chemical parameters of water samples from these two control sites (Sites 3,7) were similar and typical of coastal water quality. The results of averaging all the data are summarized in Table 4 and show that water at these two sites had comparable salinities (33.2 vs 33.6 ppt), pH (8.05 vs 8.15), dissolved oxygen (7.15 vs 6.89 mg/l) and reactive phosphorus (0.19 vs 0.15 mg/l).

The geometric mean concentrations for all bacteria in water samples from these two control sites (Sites 3, 7) are summarized in Table 4. The results show that the concentrations of enterococci (1.5 vs 1.9 CFU/100 ml), E. coli (2.1 vs 1.1 CFU/100 ml), fecal coliform (4.2 vs 2.2 CFU/100 ml), C. perfringens (0.2 vs 0.1 CFU/100 ml) and bacillus spores (0.7 to 0.9 CFU/100 ml) are relatively low and comparable in water samples from Sites 3 and 7. Thus, based on the geometric mean concentrations of five bacteria in 15 to 18 water samples, it can be concluded that the water at these two sites readily met all existing recreational water quality standards, including Hawaii's very restrictive standard of a geometric mean of 7 enterococci/100 ml. Based on the data obtained, one can conclude that the quality of water at Sites 3 and 7 is good and is unpolluted. The data support the selection of Sites 3 and Site 7 as good control sites.

Decisions on whether a site meets recreational water quality standards must follow USEPA guidelines on the frequency of analyzing water samples from a given site. Officially, the guidelines state that five water samples should be taken every six days over a 30 day period. This criteria was never achieved in this study. However, we calculated the geometric mean concentrations of all 15 or 18 water analyzed per site and compared that with the recreational standard.

Besides determining water quality based on geometric mean, the quality of water at each site should be examined to determine the number of individual water assays which exceeded the given recreational water quality standard. This kind of data (Table 5-9) can be used to compare relative quality from one site to another as a measure of sporadic pollution events. When this approach is taken, Site 3 exceeded the enterococci standard in 2 of the 15 sampling days (14 CFU/100 ml) while Site 7 exceeded the enterococci standard 3 of the 18

sampling days (range: 11 - 56 CFU/100 ml). None of the samples from Site 3 or Site 7 exceeded the old recreational water quality standard of 200 fecal coliform/100 ml. These results point out the difficulty in consistently meeting the new state of Hawaii marine recreational water quality standard of 7 enterococci/100 ml as compared to the EPA recommended standard of 35 enterococci/100 ml and especially the old fecal coliform standard.

In summary, based on averaging the measurements for various parameters in 15 to 18 water samples, the quality of water at Site 3 and Site 7 was good and acceptable for recreational use. These results support our decision in selecting Sites 3 and 7 as control sites. Although, these sites were designated as unpolluted, there were individual days when the concentrations of enterococci at these sites exceeded the 7 enterococci standard used by the State of Hawaii for marine recreational standard. Values at these sites will be used to compare similar values obtained at the selected test sites within and near Kuhio Beach.

B. Storm Drain Water Ocean Discharge Site. Site 5 represents the ocean site closest to the point where all of the storm drain water from the KSDS initially enters the ocean. As a result, Site 5 can be assumed to be the site for zone of initial dilution of the storm drain water and water samples from this site should show the greatest impact from the storm water. The expected number of indicator bacteria at Site 5 will be a function of the volume of storm drain water being discharged and the extent to which the ocean water will be able to mix and dilute the storm water. Measurements of water salinity at Site 5 can be taken as evidence of the mixing and dilution of storm drain water by ocean water.

The results of averaging all the water samples analyzed at this site are summarized in Table 4. Based on the chemical and physical parameters (salinity, pH, dissolved oxygen, reactive phosphorus), the values obtained at Site 5 was similar to that obtained from the control sites (Site 3, Site 7). These results indicate that during these 18 days of sampling, the amount of fresh water from the storm drain had been effectively diluted and mixed by ocean water to the extent that the measured chemical parameters could not determine whether water from this site was impacted by the storm drain. In these same water samples, the geometric mean concentrations of fecal indicator bacteria (enterococci: 4.5 CFU/100 ml, E. coli: 10.3 CFU/100 ml, fecal coliform: 16.9 CFU/100 ml) were definitely higher than at Sites 3 and 7 indicating a measurable impact of the storm drain water at Site 5. However, this impact was minor as the geometric mean concentrations of the fecal indicator bacteria were well below the recreational standards based on these bacteria. Geometric mean concentrations of C. perfringens (0.5 CFU/100 ml) and bacillus spores (2.0 CFU/100 ml) at this site were so low that its significance was difficult to assess.

As mentioned earlier, the same data can be assessed by examining the results of each sample to determine the frequency or the individual days when water quality at Site 5 exceeded the recreational standard. The results of the analysis of water samples for each of the individual days are tabulated in Table 5-9 and show that of all the marine water sites, Site 5 exceeded the 7 enterococci/100 ml level most frequently (6/18 water samples) with concentrations of enterococci of 7, 29, 35, 48, 53 and 107 CFU/100 ml. Thus, on individual days, the concentrations of enterococci at Site 5 was substantial indicating that on a given day,

the discharge of the storm drain water can greatly impacted on the quality of water at Site 5. The results in Table 5 also show that no enterococci were recovered on 4 of the 18 days indicating that on those days, there was no measurable impact of the storm drain discharge at Kuhio Beach.

In the interpretations of the results of water quality at Site 5, it must be recognized that good circulation of ocean current occurs at this site. Although, we previously determined that storm drain water in the KSDS contained consistently elevated concentrations of fecal indicator bacteria, the impact on the quality of ocean water at Site 5 is dependent to a large extent on the volume of the storm drain water and how well this storm water is mixed with ocean water. Based on our observation, the flow of storm water is generally low and sporadic. If the volume of storm drain water being discharged into the ocean is small, the dilution and circulation effects of ocean water will minimize the impact on the quality of the ocean water. However, when the volume of the storm drain increases such as following a rainfall event or by the discharge of sump water into the storm drain, a significant and measurable impact on the quality of water at Site 5 can be expected.

C. Sampling Sites Within Enclosed Area of Kuhio Beach.

For sampling purposes, Kuhio Beach was divided into two sections. Site 1 was the sampling site representing the body of water in Kuhio Beach which was closer to Site 5 (the storm drain discharge site) and Site 2 was the sampling site representing the half of Kuhio Beach which was farther away from Site 5. The results of concurrently analyzing the quality of the water in the storm drain and within Kuhio Beach are summarized in Table 4. Based on the averages of the chemical and physical measurements of the 15-18 marine water samples, the water samples at Sites 1 and 2 had comparable values for salinity (33.4 vs 33.4 ppt), pH (8.02 vs 7.92), and dissolved oxygen (6.44 vs 6.39 mg/l). The average reactive phosphorus value was slightly higher at Site 1 (0.034 mg/l) as compared to Site 2 (0.014 mg/l). This can only be taken as suggestive evidence that that storm water is having a measurable impact at Site 1.

The geometric mean concentrations of the five bacteria in all of the water samples are summarized in Table 4 and show similar concentrations of enterococci (2.0 vs 2.3 CFU/100 ml), E. coli (4.9 vs 6.5 CFU/100 ml), fecal coliform (12 vs 10.5 CFU/100 ml), C. perfringens (0.3 vs 0.2 CFU/100 ml) and bacillus spores (2.4 vs 2.2 CFU/100 ml) at Sites 1 and 2. Thus, based on geometric mean concentrations of fecal indicator bacteria in 15 - 18 water samples, the quality of water within Kuhio Beach is good and readily met Hawaii's strict recreational standard of 7 enterococci/100 ml.

Another way to assess the impact of the storm drain water within Kuhio Beach is to determine the frequency the individual water samples exceeded the recreational water quality standards of 7 enterococci/100 ml. The results for each of the 15 to 18 sampling days as tabulated in Tables 5-9 show that at Site 1, 3 of 18 water samples exceeded the enterococci standard with concentrations of 7, 27, and 60 CFU/100 ml. At site 2, the frequency of exceeding the enterococci standard was 4 of 18 water samples at concentrations of 7, 10, 10,

and 14 CFU/100 ml. These results indicate that on individual days, concentrations of enterococci exceeding the standard of 7 enterococci/100 ml can be expected within Kuhio Beach at Sites 1 and 2. During these same sampling days, Site 5 exceeded the enterococci standard 6 of the 18 days and three of these days correlated with the elevated concentrations observed at Sites 1 or 2. These results are suggestive that the source of the enterococci at Sites 1 and 2 is water from Site 5 (storm water).

D. Sampling Sites outside Kuhio Beach

To determine the transport of the storm water outside of the Kuhio Beach area after it has been discharged into the ocean near Site 5, two other sampling sites east and west of Site 5 were selected. Site 4 is just outside the breaker of Kuhio beach and 75 meters west of Site 5 while Site 6 is just east of Site 5. Due to the predominating wave action, water at Site 5 can be expected to be transported to Sites 4 and 6.

The average chemical parameters of water samples obtained from Sites 4 and 6 are summarized in Table 4 and show comparable values for salinity (33.4 vs 32.9 ppt), pH (8.07 vs 8.21), and dissolved oxygen (7.66 vs 7.38 mg/l). These values are similar to water samples obtained from the control sites (Sites 3, 7), indicating that the water at Sites 4 and 6 have been mixed well with ocean water. Although slightly elevated average concentrations of reactive phosphorus were observed at Site 4 (0.041 mg/l) and Site 6 (0.022 mg/1) as compared to Site 3, it was difficult to interpret this data since the average reactive phosphorus at Site 5 was low (0.015 mg/l).

The geometric mean concentrations of the five bacteria in water samples obtained at Sites 4 and 6 are summarized in Table 4. The results show that the bacterial concentrations at Site 4 were comparable to the values obtained from the control Sites 3 and 7 and were lower than at Site 6. At Site 6, slightly elevated concentrations of enterococci (3.5 CFU/100 ml), E. coli (7.0 CFU/100 ml), and fecal coliform (11 CFU/100 ml) were observed. However, the concentrations of these bacteria at Site 6 were well below the recreational water quality standard based on the concentrations of these bacteria. The concentrations of C. perfringens (0.4 vs 0.5 CFU/100 ml) and bacillus spores (1.7 vs 3.3 CFU/100 ml) at Sites 4 and 6 were low but slightly higher than the control Sites 3 and 7. These results are consistent with the observation that water from the storm drain is transported both east and west of the discharge point (Site 5). Due to the wave current, more of the storm water is transported east or away from the opening to Kuhio Beach.

Assessment of the results of individual water samples for Sites 4 and 6 are summarized in Table 5-9 and show that none of the 15 water samples from Site 4 exceeded the 7 enterococci /100 ml standard while 3/15 water samples from Site 6 exceeded this standard with concentrations of 37, 61 and 308 CFU/100 ml. The extremely high count of 308 CFU/100 ml was obtained in water sample taken the day after Hurricane Iniki and does not represent a typical day. The overall results support the conclusion made earlier that most of the storm drain water being discharged at Site 5 is transported toward Site 6 rather than Site 4.

IX. MONITORING BEACH WATER QUALITY FOR EPIDEMIOLOGICAL STUDY

One of the most serious questions related to the discharge of the Kapahulu storm drain water into the ocean water is whether this practice results in substantially increasing the incidences of diseases among swimmers at Kuhio Beach. To address this question, a 16 month long epidemiological/water quality study was initiated. In this section we report the water quality monitoring data obtained by the microbiological team in support of the epidemiological study. The results of the epidemiological findings are contained in a companion report by authored by Morens, Roll and Fujioka (KSDS-5/1994).

To support the epidemiological study, water samples from four sites were analyzed for enterococci, E. coli, fecal coliforms, C. perfringens and bacillus spores on the same days that the epidemiological team were interviewing swimmers at Kuhio Beach. Selected water samples were also analyzed for staphylococci bacteria, reactive phosphorus, pH and salinity. The primary swimming sites within Kuhio Beach (Site 1, Site 2), and the mouth of the storm drain discharge site (Site 5) were sampled on 164 days, with both morning and afternoon samples being taken on 141 of those days. Site 7, the control site, was sampled on 146 days with morning and afternoon sampled taken on 133 of those days.

A. Site 7. This popular swimming site (Queen's Beach) was selected as the control site and is characterized by absence of nearby storm drain discharge. The monthly as well as the cumulative geometric means for all the indicator bacteria in water samples obtained from this site over the sixteen month period are summarized in Table 14. The results show that the cumulative geometric means over the entire 16 month period for all of the indicator bacteria (enterococci: 1.4 CFU/100 ml, E. coli: 1.5 CFU/100 ml, fecal coliform: 2.0 CFU/100 ml, C. perfringens: 0.4 CFU/100 ml bacillus spores: 2.9 CFU/100 ml) were low. Moreover, none of the 16 monthly geometric means at Site 7 exceeded the most restrictive recreational water quality standard of 7 enterococci/100 ml. The geometric mean concentrations for enterococci were similar whether the samples were taken in the morning (1.4 CFU/100 ml) or in the afternoon (1.3 CFU/100 ml). These results indicate that the quality of water at Site 7 was excellent and suitable for recreational use.

B. Site 5. This is the site where the storm water from the Kapahulu storm drain is discharged into the ocean. It is located outside of Kuhio Beach. This area is generally not used for swimming but some swimmers have been observed to jump off the pier into the ocean water at this site and surfers do ride their boards into this area. The results of the quality of water at this site is summarized in Table 15 and show that the cumulative geometric mean over the entire 16 month period for enterococci was 7.1 CFU/100 ml or equal to the marine water recreational water quality standard. However, this standard was exceeded in 6 of the 16 months at this site. The six consecutive months (September - February) represents the rainy months of the season and most likely reflect the greater volume of storm drain water flowing into the ocean. The geometric mean for enterococci obtained from the morning samples (8.4 CFU/100 ml) was higher than the geometric mean obtained from this same site during the afternoon (6.7 CFU/100 ml).

For E. coli the cumulative geometric mean over the 16 month period was 9.4 CFU/100 ml which is higher than that of enterococcus but well below the federal fresh water recreational standard of 126 E. coli/100 ml. The geometric mean concentration of E. coli was higher during the morning (11 CFU/100 ml) than in the afternoon (8.7 CFU/100 ml). For fecal coliform, the cumulative geometric mean was 14 CFU/100 ml which was higher than that of E. coli but well below the old recreational water quality standard of 200 fecal coliform/100 ml. The geometric mean concentration of fecal coliform was higher during the morning (18 CFU/100 ml) than in the afternoon (11 CFU/100 ml). For C. perfringens the cumulative geometric mean was 0.68 CFU/100 ml which was below the 5 CFU/100 ml guideline suggested for beach water. The geometric mean concentration of this bacteria was very low and similar in the morning (0.72 CFU/100 ml) or in the afternoon (0.69 CFU/100 ml). For bacillus spores the cumulative, the morning and the afternoon geometric means were similar and ranged from 5.0 to 5.2 CFU/100 ml.

In summary, based on the cumulative geometric means of all the indicator bacteria, the water at Site 5 contained highest concentrations of fecal coliform (13.9 CFU/100 ml), followed by E. coli (9.4 CFU/100 ml), enterococci (7.1 CFU/100 ml), bacillus spores (5.2 CFU/100 ml) and lowest concentrations of C. perfringens (0.7 CFU/100 ml). The same relative concentrations of these bacteria were also measured in the storm drain (see Table 1). These results indicate that the source of the indicator bacteria at Site 5 is the storm water. Moreover, since the cumulative mean concentration of enterococci at Site 5 was equal to the marine recreational water quality standard of 7 enterococci/100 ml, it must be concluded that the discharge of storm water had a deleterious impact on the quality of water at Site 5. This impact of the storm drain water at Site 5 was more evident when the geometric mean concentrations of enterococci were exceeded during the six winter months at concentrations ranging from 8 to 30 CFU/100 ml).

C. Site 1. This is the site within the enclosed area of Kuhio Beach which is closest to Site 5 (see Figure 1). It is a popular swimming site and swimmers at this site were interviewed by the epidemiological team. The data summarizing the quality of water at this site are summarized in Table 16 and show that the cumulative geometric mean concentration of enterococci over the 16 month period was 4.9 CFU/100 ml. This was well below the recreational standard of 7 enterococci/100 ml. However, this standard was exceeded in four of the sixteen months. The four months (October to January) represented the rainy season and the same months when concentrations of indicator bacteria at Site 5 showed an increase. Thus, the evidence indicate that the source of the elevated concentrations of enterococci at Site 1 was the storm drain. This became apparent during the winter months when increase rainfall results in increasing the volume of storm water and indicator bacteria being discharged into Site 5. Some of the water at Site 5 which is contaminated with indicator bacteria such as enterococci is then transported to Site 1. The cumulative geometric mean concentrations of enterococci obtained from morning samples (3.9 CFU/100 ml) was slightly lower than the geometric mean obtained from this same site during the afternoon (4.9 CFU/100 ml).

For E. coli the cumulative geometric mean over the 16 month period was 6.6 CFU/100 ml, well below the fresh recreational water quality standard of 126 E. coli/100 ml and none of

the monthly geometric mean approached this standard. The geometric mean concentrations of E. coli was higher in the morning samples (7.3 CFU/100 ml compared to the afternoon samples (5.9 CFU/100 ml). For fecal coliform, the cumulative geometric mean was 9.2 CFU/100 ml but well below the old recreational standard of 200 fecal coliform/100 ml and none of the monthly geometric mean approached this standard. Higher geometric mean concentrations of fecal coliform were recovered from this site in the morning samples (10.6 CFU/100 ml) as compared to the afternoon samples (9.3 CFU/100 ml). For C. perfringens, the geometric mean concentrations in all samples, in the morning and afternoon samples were approximately 0.5 CFU/100 ml which was well below the 5 CFU/100 ml guideline suggested for beach water. For bacillus spores, the cumulative geometric mean was 4.9 CFU/100 ml with geometric mean of 4.6 CFU/100 ml in the morning and 5.0 CFU/100 ml in the afternoon.

In summary, based on the cumulative geometric mean concentrations, Site 1 contained highest concentrations of fecal coliform (9.2 CFU/100 ml) followed by E. coli (6.6 CFU/100 ml), followed by enterococci and bacillus spores (4.9 CFU/100 ml) and finally by C. perfringens at only 0.5 CFU/100 ml. This is the same relative concentrations of indicator bacteria as was observed at Site 5 and provide additional evidence that the source of indicator bacteria at Site 1 was the storm drain water. The overall quality of water at Site 1 met the stringent Hawaii marine recreational water quality standard of 7 enterococci/100 ml. However, this standard was exceeded during four of the sixteen months indicating that the storm drain does impact on the quality of water at Site 1.

D. Site 2. This site is also within the enclosed area of Kuhio Beach but west of Site 1 and farther away (100 meters) from Site 5. As with Site 1, Site 2 is heavily used by swimmers and swimmers at this site were also interviewed by the epidemiological team. The data summarizing the quality of water at this site are summarized in Table 17 and show that the cumulative geometric mean concentration of enterococci over the entire 16 month period was 3.5 CFU/100 ml. This was well below the level observed at Site 1 and well below the Hawaii marine recreational standard of 7 enterococci/100 ml. However, this standard was exceeded in three of the sixteen monthly means. These three months (September, October, November) represent the rainy season and are the same months when elevated concentrations of enterococci were recovered from Site 5 and Site 1. These results indicate that the source of elevated enterococci at Site 2 is the same source (storm water) which are affecting Sites 5 and Site 1. The results indicate that the indicator bacteria originate from storm water which is discharged into the ocean at Site 5 and transported to Sites 1 and then to Site 2. The cumulative geometric mean concentration of enterococci obtained in the morning (3.5 CFU/100 ml) was similar to that obtained from afternoon (3.3 CFU/100 ml) samples.

For E. coli, the cumulative geometric mean over the 16 month period was 4.5 CFU/100 ml, well below the fresh recreational water quality standard of 126 E. coli/100 ml and none of the monthly geometric means exceeded this standard. The geometric mean concentrations of E. coli during the morning samples (5.9 CFU/100 ml) was higher than in the afternoon samples (2.8 CFU/100 ml). For fecal coliform, the cumulative geometric mean was 7.0 CFU/100 ml, well below the old standard of 200 fecal coliform/100 ml and none of the monthly geometric mean approached this standard. Higher geometric mean concentrations of

fecal coliform were recovered from the morning samples (8.8 CFU/100 ml) as compared to the afternoon samples (4.8 CFU/100 ml). For C. perfringens, the cumulative geometric mean, as well as the mean recovered from the morning and afternoon samples were low and very similar ranging form 0.4 to 0.5 CFU/100 ml. This was well below the 5 CFU/100 ml guideline for beach waters. For bacillus spores, the cumulative geometric mean was 5.6 CFU/100 ml with geometric mean of 4.5 CFU/100 ml in the morning samples and 6.1 CFU/100 ml in the afternoon samples.

In summary, based on cumulative geometric mean concentrations, Site 2 contained highest concentrations of fecal coliform (7.0 CFU/100 ml) followed by bacillus spores (5.6 CFU/100 ml), followed by E. coli (4.5 CFU/ 100 ml), followed by enterococci (2.5 CFU/100 ml) and finally by C. perfringens at 0.4 CFU/100 ml. Thus, the relative concentrations of the fecal indicators at Site 2 were similar to that at Site 1 and Site 5 but the measured concentrations were lower. These results provide additional evidence that the source of indicator bacteria at Site 2 was the storm drain water. Moreover, that the quality of water at Site 2 was better than at Site 1 and generally met the Hawaii marine recreational water quality standard. However, this standard was exceeded during three of the sixteen months. As with Site 1, the months with the elevated enterococci represented the rainy season and indicate that the source of the enterococci is storm water which initially impacts Site 5 and is then transported to Site 1 and then to Site 2. The lowest concentrations of indicator bacteria at Site 2 most likely represent greater dilution and some inactivation as the indicator bacteria is transported from Site 5 to Site 2.

X. SOURCES OF INDICATOR BACTERIA RECOVERED FROM KUHIO BEACH

A. Sewage as source of indicator bacteria. Presence of fecal indicator bacteria in water initially suggests that the source of the fecal indicator bacteria is sewage. However, a sanitary survey of the Kuhio Beach area indicated that sewage was not being discharged into the waters at Kuhio Beach. It should be noted that based on USEPA studies (Cabelli, 1983, Dufour, 1984), there will be a predictable number of diarrheal diseases among swimmers as the concentrations of fecal indicator bacteria from a sewage source increases.

B. Storm drain as source of indicator bacteria. The storm drain water from the KSDS was designed to be discharged into the ocean near Kuhio Beach near Site 5. The results of this study have documented the presence of high concentrations of fecal indicator bacteria in the storm drain water of the KSDS and discharge of these bacteria at Site 5 near the Kuhio Beach. The same relative concentrations of indicator bacteria observed in the storm drain was also observed at Sites 5, Site 1 and Site 2. Moreover, the monthly geometric means of these fecal indicator increased at all three of these sites during the rainy season. These results support the known effects of rainfall which washes more fecal indicator bacteria into the storm drain, increases the volume of water in the storm drain, and thereby increasing the load of pollution at Site 5.

Based only on the expected concentrations of enterococci at Sites 5, 1 and 2, it may be prudent to designate these sites unsuitable for swimming during the winter months or anytime it rains. However, the increase in the concentrations of enterococci at Sites 5, 1 and 2 does not automatically mean there will be an increase in disease incidences as predicted by the USEPA studies. Those studies which established the enterococci standard documented that at recreational sites where point source of sewage discharge were present, the incidences of diseases increased as the concentrations of enterococci in the water increased. However, in a more recent USEPA study, Calderon et al (1991) determined that increase in enterococci in water from non-point source without a source of sewage did not result in the corresponding increase of diarrheal diseases among swimmers using that water. Since the source of indicator bacteria at Sites 5, 1 and 2 is from the storm drain, which represents non-point source rather than a sewage source, the conditions more closely approximates the study of Calderon et al (1991) rather than the Cabelli study (1983). The very low concentrations of C. perfringens at Sites 5, 1 and 2 also indicate that sewage is not a major source of enterococci at these sites. These results suggest that the concentrations of enterococci at Kuhio Beach may not be related to increased concentrations of swimming associated diarrheal diseases. However, only the results of an epidemiological study can resolve the health risks related to the concentrations of enterococci at Kuhio Beach.

C. Swimmers as sources of staphylococci bacteria. In a previous study, Charoenca and Fujioka (1993) reported a correlation between the number of swimmers at Kuhio Beach and the concentrations of staphylococci bacteria in the water. Swimmers were concluded to be sources of staphylococci bacteria in waters at Kuhio Beach. Moreover, the increase in staphylococci in the water at Kuhio Beach was correlated with the increase in numbers of reported skin infections among children swimming at this beach.

In the present epidemiological study, illness due to diarrheal diseases as well as skin diseases were asked. An attempt was made to determine the contribution of staphylococci by swimmers by analyzing six water samples from Sites 1, 2, 5 and 7 for concentrations of staphylococci. The results of the presumptive staphylococci concentrations are summarized in Table 18 and show that Site 1 and Site 2, which consistently had higher numbers of bathers contained the highest geometric mean concentrations of staphylococci. Site 1 had a geometric mean of 643 CFU/100 ml in the morning samples and 402 CFU/100 ml in the afternoon samples. All 12/12 (100%) of samples from Site 1 exceeded the suggested guideline of not more than 100 staphylococci/100 ml (Favero et al., 1964). Site 2 had the next highest geometric mean concentrations of staphylococci with 139 CFU/100 ml during the morning samples and 84 CFU/100 ml during the afternoon samples. Fifty percent (6/12) of the water samples from Site 2 exceeded 100 staphylococci/100 ml. At Site 5, the mean concentrations of staphylococci was 39 CFU/100 ml in the morning and 41 CFU/100 ml in the afternoon with only 1/8 or 8% of the samples exceeding the 100 staphylococci/100 ml level. Site 7, the control site had the lowest geometric mean concentrations of staphylococci with 21 CFU/100 ml in the morning samples and 26 CFU/100 ml in the afternoon samples. However, 3/12 (25%) of the samples at this site exceeded the 100 staphylococci/100 ml guideline.

To determine if swimmers were sources of staphylococci, a statistical analysis based on correlation coefficient indicated that the number of swimmers were correlated to the staphylococci concentrations in the water at sites 1 and 2. The results (Table 20) show a significant correlation at Site 1 (R=0.823, p=0.0443) in afternoon samples, and at Site 2 (R=0.921, p=0.009) in morning samples. These results and the observation of lower concentrations of staphylococci at Site 5 and higher concentrations of staphylococci at Sites 1 and 2 support the conclusion that swimmers do contribute to levels of staphylococci bacteria in the ocean water.

D. Sand as source of indicator bacteria. In previous studies, Fujioka and Oshiro (1990) reported that sand was a major source of indicator bacteria for the water at Hanauma Bay. To determine whether sand is a major source of indicator bacteria at Kuhio Beach, dry and wet sand samples from Sites 1, 2, and 7 were analyzed for the various indicator bacteria on three different days. The results of the sand analysis are summarized in Table 19 and show that dry sand samples contain much higher concentrations of indicator bacteria than wet samples. These results are similar to that observed in earlier Hanauma Bay study and reflect the observation that soil in the sand is the source of the bacteria and wet sand has been washed free of soil by the surf. Dry sand contain more soil and therefore more indicator bacteria.

At Site 1, dry sand contained geometric mean concentrations of 1,323 CFU/100 g of enterococci, 297 CFU/100 g of E. coli, 3,088 CFU/100 g of fecal coliform, 23 CFU/100 g of C. perfringens and 2,837 CFU/100 g of bacillus spores. At Site 2, dry sand contained geometric mean concentrations of 3,679 CFU/100 g of enterococci, 4.4 CFU/100 g of E. coli, 63 CFU/100 g of fecal coliform, 72 CFU/100 g of C. perfringens and 1,054 CFU/100 g of bacillus spores. At Site 7, dry sand contained geometric mean concentrations of 2.2 CFU/100g of enterococci, 1.1 CFU/100 g of E. coli, 0 CFU/100 g of fecal coliform, 6.2 CFU/100 g of C. perfringens and 88 CFU/100 g of bacillus spores.

The results of sand analysis showed that more indicator bacteria are present in the sand at Kuhio Beach (Site 1, 2) than at Site 7. Based on the study conducted at Hanauma Bay, the source of the bacteria in the sand is soil. This is supported by the observation that the sand at Kuhio Beach visibly contains more dirt than the sand at Site 7. Two factors may increase the soil content of the sand at Kuhio Beach. First, there are more swimmers who will track more soil to the sand at Kuhio Beach. Second, due to the breakers, wave action at Kuhio Beach is minimized and the soil in the sand cannot be washed out to sea as can be expected at Site 7. Thus, the source of some of the indicator bacteria in the water at Kuhio Beach must come from the soil in the sand. However, the contribution of sand to the actual numbers of fecal indicator recovered from the waters in Kuhio Beach is not known.

E. Swimmers or people as sources of indicator bacteria. Swimmers are known sources of indicator bacteria and more importantly known sources of various types of water borne pathogens. In an enclosed area such as Kuhio Beach, swimmers can be expected to contribute to the pollution load in the beach water. The results of the staphylococci study showed that swimmers at Kuhio Beach are contributing to the high levels of staphylococci at Kuhio

Beach. However, the source of staphylococci is the skin of people whereas the source of fecal indicator bacteria from people is feces.

To determine the impact of swimmer density to the concentrations of fecal indicator bacteria, a correlation coefficient assessment was made. The results of this assessment indicated no correlation between density of swimmers and excessive concentrations of indicator bacteria in the waters at Kuhio Beach. One possible explanation for this finding is that the contribution of fecal indicator bacteria from swimmers are much less than the contribution of these indicator bacteria from the storm drain. This condition was supported by the following observations. First, the concentrations of fecal indicator was higher at Site 5 than at Sites 1 and 2. Second, evidence was obtained that the source of the indicator bacteria recovered from water samples at Kuhio Beach was the storm drain water being discharged at Site 5. These results show that the contribution of indicator bacteria in Kuhio Beach is predominantly controlled by the storm drain and masks the lower contribution of indicator bacteria from other sources such as people and beach sand.

Acknowledgments: We thank Stacey Paul, Khartini Luther, Tuamasaga Unutoa and Pritty Borthakur for their assistance in the microbiological analysis. We are also grateful to Lloyd Shimazu and Henry Gee for their help with the dye study and Bunnie Yoneyama who was instrumental in the laboratory throughout the entire study.

REFERENCES

American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1989. Standard Methods for the Examination of Water and Wastewater, 17th edition. American Public Health Association, Washington D.C.

Bisson, J.W., V.J. Cabelli. 1979. Membrane filter enumeration method for Clostridium perfringens. *Appl Environ Microbiol.* 37:55-66.

Cabelli, V.J. 1983. Health effects criteria for marine recreational waters. USEPA Report EPA-600/1-80-031.

Calderon, R.L., E. W. Mood, A.P. Dufour. 1991. Health effects of swimmers and nonpoint sources of contamination water. *International Journal of Environmental Health Research.* 1:21-31

Charoenca, N., R. Fujioka. 1991. Assessment of Staphylococcus bacteria in Hawaii's marine recreational waters. *Wat Sci Tech.* 27:283-289.

Chun, M.J., R.H.F., Young, G.K. Anderson. 1972. Wastewater Effluents and Surface Runoff Quality. Water Resources Research Center Tech. Report No. 63.

Davis, E.M. 1979. Maximum Utilization of Water Resources in Planned Community Bacterial Characteristics of Stormwaters in Developing Rural Areas. USEPA Report EPA-600/2-79-050f.

DOH, State of Hawaii. 1992. Chapter 11-54 Hawaii Administrative Rules on Water Quality Rules.

Dufour, A.P. 1984. Health effects criteria for fresh water. USEPA Report EPA600/1-84-004.

Dutka, B.J. 1975. Coliforms are an inadequate index of water quality. *J Environ Health.* 36:39-46.

Favero, M.S., C.H. Drake, C.B. Randall. 1964. Use of staphylococci as indicator of swimming pool pollution. *Public Health Reports.* 79:61-70.

Fujioka, R.S., L.K. Shizumura. 1985. Clostridium Perfringens, a reliable indicator of stream water. *J Water Poll Cont Fed.* 57:986-992.

Fujioka, R.S., K. Tenno, S. Kansako. 1988. Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams. *Toxicicity Assessment.* 3:613-630.

Fujioka, R.S. 1990. Evaluation of Clostridium perfringens as a suitable indicator for recreational water quality standard. Project Completion Report. ASO LOG No. 89-355.

Hardina, C.M., R.S. Fujioka. 1991. Soil: The environmental source of E. coli and enterococci in Hawaii's streams. *Environ. Toxicology.* 6:185-195.

Harrigan, J. 1991. Bacteriology on the Relationship Between Monitoring Data and the Marine Recreational Waters Standard Part A: Waikiki Beach, Oahu. Internal Department of Health Document.

Hazen, T.C. 1988. Fecal coliforms as indicators in tropical waters: A review. *Toxicity Assessment: International Journal.* 3:461-477.

Oliveri, V.P., K.W. Cornelius, K. Kawata, J. E. Smith. 1977. Microorganisms in Urban Stormwater. USEPA Report EPA-600/2-77-087.

Oshiro, R. 1990. Sand, soil, and pigeon dropping: Sources of indicator bacteria in the waters at Hanauma Bay beach, Hawaii. Paper presented at the 90th Annual meeting of American Society for Microbiology.

Scott, W.J. 1932. Survey of Connecticut's Shore Bathing Waters. *Public Health Engr.* 19:316.

Sloat, S., C. Zeil. 1987. The use of indicator organisms to assess the safety of public water. HACH Technical Center for Applied Analytical Sciences.

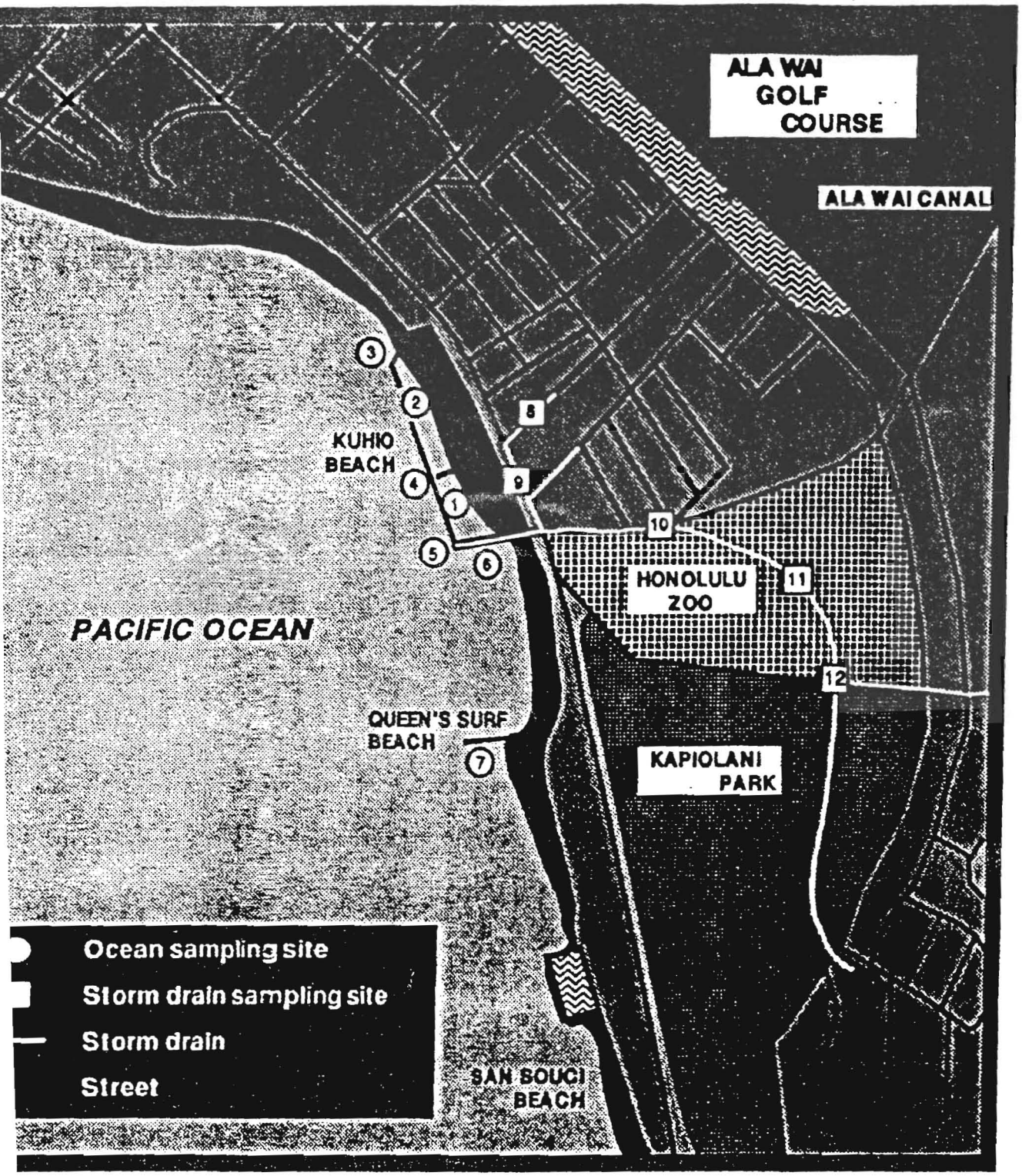
Smart, P.L., I.M.S. Laidlaw. 1977. An Evaluation of Some Fluorescent Dyes for Water Tracing. *Water Resources Research.* 13:15-33.

U.S. Environ. Prot. Agency. 1976. Quality criteria for water (EPA/440/9-76/023). Washington D.C.: US EPA.

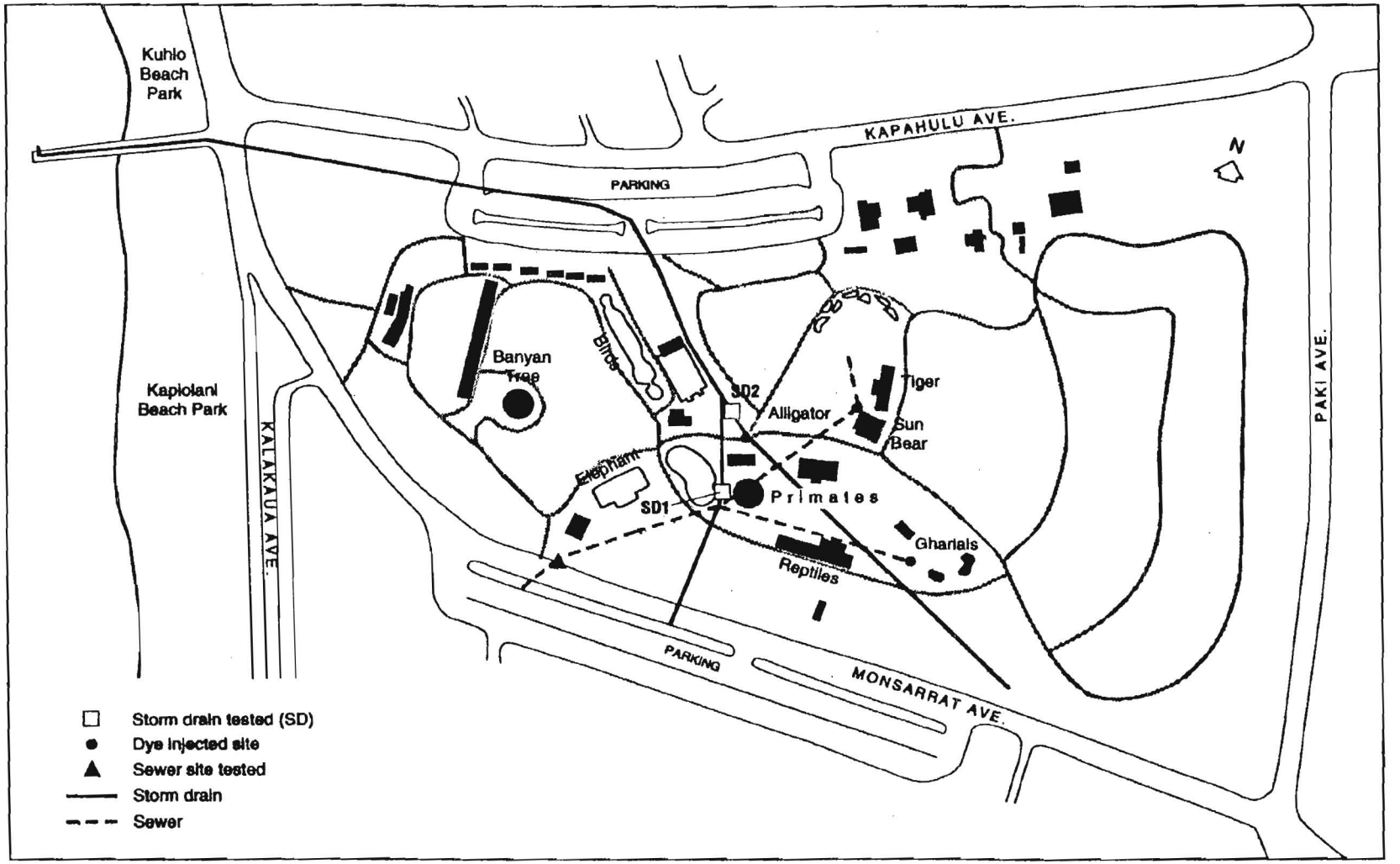
U.S. Environ. Prot. Agency. 1984. Water quality criteria: Request for comments. *Fed Reg.* 51:8-12-8016.

U.S. Environ. Prot. Agency. 1986. Ambient water quality criteria for bacteria - 1986 (EPA440/5-84-002). Washington D.C.: US EPA.

Young, R. 1978. Urban storm water runoff in Hawaii. International Symposium on Urban Storm Water Management. University of Kentucky, Lexington, Kentucky. July 24-27.



ure 1. Sample Sites for Kapahulu Storm Drain System/Kuhio Beach Study



SOURCE: Modified from Parks and Recreation, Honolulu Zoo Master Plan.

FIGURE 1. Study sites, Honolulu Zoo, September 1993

Table 1. Parameter Levels for Kapahulu Storm Drain System Sites

	Storm Drain Sites				
	Hotel Tributary		Urbanized Tributary		
Parameter	8	9	12	11	10
[a]Salinity (ppt)	**0.9**	**13.8**	**11.8**	**15.2**	**16.1**
N	18	18	18	18	18
Min.	0	*0	4	0	4
Max.	10	24	20	28	27
[a]pH	**7.74**	**7.73**	**7.77**	**7.81**	**7.77**
N	9	9	9	9	9
Min.	7.54	7.20	7.60	7.62	7.46
Max	8.04	7.93	7.94	7.92	7.99
[a]Dissolved Oxygen (mg/L)	**3.3**	**5.4**	**2.2**	**3.3**	**3.5**
N	17	17	17	16	17
Min.	1.5	4.0	0	*2.2	2.5
Max.	6.3	7.5	5.5	7.3	4.7
[a]Reactive Phosphorus (mg/L)	**1.26**	**.331**	**.757**	**.188**	**.174**
N	18	18	18	18	18
Min.	.078	.043	.328	.058	.047
Max.	7.13	.409	1.53	.497	*.300
[b]Enterococci (CFU/100ml)	**890**	**241**	**3975**	**376**	**1510**
N	18	18	18	18	18
Min.	40	0	124	16	80
Max.	*404000	20000	**204800	*22000	123600
[b]E. coli (CFU/100ml)	**9291**	**671**	**6270**	**572**	**2089**
N	18	18	18	18	17
Min.	760	0	560	16	200
Max.	*372000	*72400	*264000	*64800	*72400
[b]Fecal Coliform (CFU/100ml)	**24081**	**1492**	**5961**	**851**	**2145**
N	18	18	18	18	18
Min.	1400	0	135	20	40
Max.	1936000	*25200	*480000	*16000	*94800
[b]C. perfringens (CFU/100ml)	**11**	**4.7**	**147**	**58**	**31**
N	18	18	18	18	18
Min.	0	0	0	4	7
Max.	264	70	3120	372	152
[b]Bacillus spores (CFU/100ml)	**24**	**70**	**4.0**	**18**	**33**
N	18	18	18	18	18
Min.	0	4	0	0	4
Max.	696	840	320	320	*396

*Samples taken day after Hurricane Iniki

**Estimated from maximum CFU countable

[a]average

[b]geometric mean

Table 2. Fluorescein Levels (ppm) in Zoo Sewer Line and Two Storm Drains During Initial Dye Test (9/30/93) and Follow Up Analysis of Background Levels (10/7/93)

	Initial Dye Study Fluorescein Levels (9/30/93)			Background Fluorescein Levels (10/7/93)		
Time (min.)	Sewer Line	SD1	SD2	SD1	SD2	Sewer Line
0	0.000662	3.14E-05	0.000226	0.005233	0.001255	*
10	*	*	*	NTD	NTD	*
15	0.000662	2.24E-05	0.000226	*	*	*
17	0.045567	*	*	*	*	*
20	0.810762	*	*	0.011126	NTD	*
25	26.01709	*	*	*	*	*
30	50.67568	2.24E-05	9.28E-05	0.012108	NTD	*
35	98.25786	*	*	*	*	*
40	478.5828	*	*	0.00376	NTD	*
45	535.8551	0.000113	9.28E-05	*	*	*
50	375.4928	*	*	0.005724	NTD	*
55	306.7661	*	*	*	*	*
60	62.74907	9.48E-05	0.00036	0.010635	0.001255	*
65	2.380394	*	*	*	*	*
70	2.838572	*	*	0.009653	2.77E-05	*
75	3.182206	4.96E-05	9.28E-05	*	*	*
80	3.411295	*	*	0.012108	0.000846	*
85	2.494939	*	*	*	*	*
88	329.675	*	*	*	*	*
90	4.098562	4.05E-05	0.002098	0.012599	0.000437	*
94	0.558764	*	*	*	*	*
100	*	*	*	0.008671	2.77E-05	*
110	*	*	*	0.011617	0.001255	*
120	*	*	*	0.008671	0.000846	.000112

*Readings not taken
NTD-Not Detected
Note: Fluorescein dye introduced into sewer line at time = 5 min. and 75 min. on 9/30/93

Table 3 . Levels of Fecal Indicator Bacteria in Kapiolani Park Soil

	MPN Index/g		
Soil Sample Site	Fecal Coliform	Fecal Streptococcus	C. perfringens
1* Within Zoo	1,100	≥16,000	500
2* Outside Zoo	≥ 16,000	≥16,000	300
3 Private Home	1,300	16,000	90

*Frequented by high populations of pigeons

Table 4. Parameter Levels for Kuhio Beach Study Sites

	Ocean Sites						
	Within Kuhio Beach		Near Mouth of KSDS			Contol Sites	
Parameter	1	2	4	5	6	3	7
[a]Salinity (ppt)	**33.4**	**33.4**	**33.4**	**33.0**	**32.9**	**33.2**	**33.6**
N	15	16	14	16	13	15	16
Min.	30	*32	*32	30	*32	*32	*32
Max.	34	34	34	34	34	34	34
[a]pH	**8.02**	**7.92**	**8.07**	**8.10**	**8.21**	**8.05**	**8.15**
N	9	9	6	9	6	6	9
Min.	7.26	6.83	7.54	7.46	8.02	7.79	8.06
Max	8.24	8.11	8.31	8.31	8.37	8.25	8.27
[a]Dissolved Oxygen (mg/L)	**6.44**	**6.39**	**7.66**	**6.87**	**7.38**	**7.15**	**6.89**
N	17	17	14	17	13	14	17
Min.	2.0	5.5	6.1	6.0	6.7	6.1	6.0
Max.	8.3	8.0	9.4	8.2	*8.0	9.0	10.0
[a]Reactive Phosphorus (mg/L)	**.034**	**.014**	**.041**	**.015**	**.022**	**.019**	**.015**
N	17	18	15	18	15	14	18
Min.	0	0	0	0	0	0	0
Max.	.167	.057	.234	.051	.079	.103	.037
[b]Enterococci (CFU/100ml)	**2.0**	**2.3**	**1.6**	**4.5**	**3.5**	**1.5**	**1.9**
N	18	18	15	18	15	15	18
Min.	0	0	0	0	0	0	0
Max.	60	*13.6	*6.4	107	*308	*13.6	56
[b]E. coli (CFU/100ml)	**4.9**	**6.5**	**3.1**	**10.3**	**7.0**	**2.1**	**1.1**
N	18	18	15	18	15	15	18
Min.	0	0	0	0	0	0	0
Max.	*148	240	*180	*552	*1456	*84	*104
[b]Fecal Coliform (CFU/100ml)	**12.0**	**10.5**	**6.0**	**16.9**	**10.9**	**4.2**	**2.2**
N	18	18	15	18	15	15	18
Min.	0	0	0	0	0	0	0
Max.	*292	820	*360	*776	*1984	*124	*92
[b]C. perfringens (CFU/100ml)	**0.3**	**0.2**	**0.4**	**0.5**	**0.5**	**0.2**	**0.1**
N	18	18	15	18	15	15	18
Min.	0	0	0	0	0	0	0
Max.	14.7	*2.4	4.8	5.6	*4	*0.8	*0.8
[b]Bacillus spores (CFU/100ml)	**2.4**	**2.2**	**1.7**	**2.0**	**3.3**	**0.7**	**0.9**
N	18	18	15	18	15	15	18
Min.	0	0	0	0	1	0	0
Max.	263	109	520	94	*37	786	*39

*Samples taken day after Hurricane Iniki

[a] average

[b] geometric mean

Table 5. Enterococcus Levels (CFU/100ml) in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
6/8/92	0.8	0.8	0.8	0	0	0	0	920	384	560	76	376
6/17/92	0	1.6	1.6	1.6	0	0.8	1.6	204	3640	1440	152	2080
6/29/92	0	0	0	0	0	0	0.8	1360	0	640	128	1320
7/8/92	0	4.8	0.8	3.2	0	0.8	11.2	1160	9600	123600	2048	*204800
7/15/92	4.8	0	1.6	0	1.6	1.6	0	5400	268	2200	4000	124
7/22/92	0.8	0	0	0	0.8	1.6	3.2	1720	112	84	300	2560
7/29/92	0	0.8	0.8	3.2	4	4	4	3720	1000	600	104	2640
8/11/92	0	4	0	0	0.8	0.8	0.8	520	80	80	20	2360
8/18/92	2.4	6.7	13.6	4	2.4	4	27	112	1040	2360	1440	12000
8/26/92	7.2	9.6	0.8	12	2.4	1.6	0	264	124	132	84	1040
9/12/92	3.2	6.4	13.6	6.4	53	308	2.4	404000	3720	45200	22000	72800
10/7/92	0.8	13.6	0	2.4	1.6	0.8	0	112	20000	1240	16	27600
10/26/92	60	0.8	0.8	2.4	107	37	0	112	128	3200	760	3880
11/10/92	27	9.6	ND	ND	29	ND	0.8	1560	524	1000	1000	5320
12/14/92	5.6	4.8	ND	ND	35	ND	1.6	1440	18	1840	256	1600
1/25/93	5.6	1.6	8	4	48	61	0.8	40	0	1440	300	2560
3/22/93	0	0	2.4	0	7.2	0.8	56	260	0	1240	1040	2520
5/17/93	0	2.4	ND	ND	4.8	ND	0.8	6280	19200	15600	1000	27200
GM	1.9547	2.296	1.4815	1.5529	4.4597	3.4884	1.8728	890.1187	240.7206	1510.266	375.638	3975.317

*Estimated from maximum CFU countable
ND-Not Done
GM-Geometric Mean

Table 6. E. coli Levels (CFU/100ml) in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
6/8/92	0	0	0	0.8	2	0.8	0	19200	800	360	24	560
6/17/92	0.8	10.4	0	1.6	46	0.8	0	53200	37600	1320	116	1200
6/29/92	1.6	4	21	1.6	0.8	0.8	0	6800	0	240	108	3240
7/8/92	0.8	4.8	3.2	0	1.6	18	6.4	5600	*1840	*18400	*1840	*184000
7/15/92	0	2.4	0	0	0.8	2.4	0	12800	720	200	200	6000
7/22/92	2	240	4	0	0	0	0	4000	344	2160	2000	2700
7/29/92	1.6	0.8	0	3.2	74	8	0	4000	1520	ND	104	2040
8/11/92	1.6	0	0	0	0	0.8	0	2400	416	192	1320	636
8/18/92	4.8	4.8	5.6	6.4	0	7.2	0.8	9600	2040	8480	6800	8000
8/26/92	33	2.4	1.6	84	24	2.4	0	18000	352	344	104	4880
9/12/92	148	74	84	180	552	1456	104	372000	56800	72400	64800	264000
10/7/92	0	2.4	0	0	28	8.8	1.6	1600	920000	920	16	248000
10/26/92	42	4.8	3.2	6.4	38	31	1.6	760	304	20000	720	3880
11/10/92	56	13.6	ND	ND	40	ND	22	22400	1800	1760	1280	6400
12/14/92	32	15.2	ND	ND	27	ND	0.8	4800	16	15600	320	1120
1/25/93	29	6.4	4	16.8	44	65	0.8	5160	0	2120	2200	11600
3/22/93	0.8	0	0.8	0.8	13.6	5.6	0	72800	0	1320	1120	1640
5/17/93	2.4	232	ND	ND	8	ND	0	4400	256800	5200	2320	19600
GM	4.8978	6.5118	2.117	3.0674	10.329	6.9797	1.1129	9291.053	671.4196	2088.987	571.5634	6270.052

*Estimated from maximum CFU countable
ND-Not Done
GM-Geometric Mean

Table 7. Fecal Coliform Levels (CFU/100ml) in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
6/8/92	10.4	2.4	0	5.6	1.6	1.6	0	6520	760	520	32	840
6/17/92	3.2	7.2	2.4	2.4	112	2.4	0	143000	40400	1040	137	4160
6/29/92	4	5.6	31	2.4	0.8	1.6	0	8000	80	280	152	135
7/8/92	3.2	8.8	8	1.6	19.2	24	8.8	11200	26720	*26720	*2762	*2762
7/15/92	6.4	3	1.6	0.8	0	3.2	0	40000	680	840	13440	5000
7/22/92	23	820	12.8	2.4	2.4	0	0.8	8000	280	1600	1680	4000
7/29/92	4.8	0	0	5.6	96	30	0.8	116000	1600	40	508	6000
8/11/92	8	0.8	0.8	3.2	0	2.4	0.8	9600	400	240	92	1040
8/18/92	18.7	6.7	8	6.4	8	15.2	3.2	54000	3360	10000	3240	25600
8/26/92	28	8	3.2	116	30	5.6	0.8	1936000	9360	440	304	4880
9/12/92	292	64	124	360	776	1984	92	236000	136000	94800	160000	480000
10/7/92	3.2	11.2	0.8	1.6	24	16.8	0	10000	2520000	4320	20	232000
10/26/92	82	8	7	10.4	71	45	1.6	1400	416	23200	1240	6260
11/10/92	47	32	ND	ND	55	ND	24	39600	1600	1880	2080	18800
12/14/92	52	12.8	ND	ND	43	ND	1.6	4800	40	18000	480	1280
1/25/93	14.4	8.8	6.4	13.6	71	91	1.6	5640	0	1800	1800	8000
3/22/93	0	0.8	0.8	0	12	2.4	42	84000	0	840	1680	1640
5/17/93	4	272	ND	ND	9.6	ND	0.8	8000	564000	8000	2160	20000
GM	11.961	10.457	4.2146	5.9804	16.843	10.914	2.2176	24081.33	1492.144	2145.007	850.6729	5960.95

*Estimated from maximum CFU countable
ND-Not Done
GM-Geometric Mean

Table 8. C. perfringens Levels (CFU/100ml) in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
6/8/92	0.8	0	0	0	0	0	0	4	16	20	72	132
6/17/92	0	0	0.8	0	0	0	0	0	1	41	14.4	46
6/29/92	0	0	0	0	0	0	0	0.8	0	18.4	35	*992
7/8/92	0	0	0	0	0	0	0	30	16	39	76	336
7/15/92	0	0	0	0	0	0	0	52	6.4	19	16.8	0
7/22/92	0	0	0	0.8	0.8	0	0	20	20	20	4	272
7/29/92	0	0	0	1.6	0	0	0	264	2.4	29	41	112
8/11/92	0	0	0	0	0	0.8	0	6.4	1.6	7	18.4	19.2
8/18/92	14.7	8	0	4.8	5.6	0.8	0	23	43	24	87	384
8/26/92	0	0	0	0	0	0	0	40	0	16	52	408
9/12/92	0	2.4	0.8	1.6	1.6	4	0.8	6.4	4	148	320	680
10/7/92	0	0	0	0	0	0	0	40	52	19.2	20	368
10/26/92	0.8	0.8	0	0.8	1.6	3.2	0.8	0	1.6	20	65	228
11/10/92	0	0	ND	ND	0	ND	0	10	1.6	20	103	920
12/14/92	0	0	ND	ND	0.8	ND	0	0.8	7.2	47	76	104
1/25/93	1.6	0	0.8	0	0.8	1.6	0	12	0	152	180	3120
3/22/93	0	0	0.8	0	2.4	1.6	0	12	0	45	372	1.6
5/17/93	0	0	ND	ND	0	ND	0	72	70	75	328	128
GM	0.3118	0.2495	0.1697	0.3812	0.4578	0.5049	0.0675	11.34598	4.667863	30.6214	58.34726	147.2446

*Estimated from maximum CFU countable
ND-Not Done
GM-Geometric Mean

Table 9. Bacillus spore Levels (CFU/100ml) in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
6/8/92	0	1	0	0	2	1	1	2	176	28	2	0
6/17/92	3	6	2	5	2	2	1	35	208	15	17	3
6/29/92	12	4	4	520	94	1	0	31	73	25	27	4
7/8/92	137	4	28	0	14	4	32	696	272	64	28	2
7/15/92	263	56	239	36	8	3	33	44	76	12	312	0
7/22/92	6	3	3	3	0	21	21	80	197	37	320	0
7/29/92	5	6	12	24	3	10	7	8	143	18	13	0
8/11/92	1	3	7	1	3	10	1	4	172	40	11	1
8/18/92	0	1	0	1	5	3	7	0	80	17	5	2
8/26/92	4	109	786	80	2	2	3	12	72	27	14	3
9/12/92	26	33	68	36	37	37	39	212	82	396	63	49
10/7/92	6	8	3	4	4	9	3	340	840	39	0	320
10/26/92	5	2	1	10	9	16	2	14	16	43	34	92
11/10/92	18	3	ND	ND	8	ND	1	696	17	27	28	0
12/14/92	19	4	ND	ND	9	ND	4	3	34	40	81	101
1/25/93	2	2	6	2	2	11	2	0	20	4	16	7
3/22/93	2	0	2	0	5	12	0	0	13	41	0	0
5/17/93	10	3	ND	ND	4	ND	2	1144	4	107	23	1
GM	7.2866	5.0215	6.4015	6.7737	5.5639	6.231	3.8474	24.28646	69.48614	32.46223	18.35102	3.97676

ND-Not Done
GM-Geometric Mean

Table 10. Reactive Phosphorus (mg/l) in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
6/8/92	NTD	0.006	NTD	NTD	NTD	NTD	NTD	0.629	0.2	0.116	0.223	0.5
6/17/92	0.033	NTD	NTD	0.011	0.051	NTD	0.06	7.13	1.9	0.107	0.094	0.602
6/29/92	0.006	0.011	NTD	0.234	0.025	NTD	0.037	1.39	0.409	0.119	0.092	0.525
7/8/92	0.082	0.019	NTD	0.059	0.017	0.039	0.02	0.305	0.148	0.112	0.198	1.008
7/15/92	0.149	0.002	0.103	0.038	0.008	0.058	NTD	2.912	0.36	0.126	0.199	1.168
7/22/92	0.167	0.057	0.06	0.01	0.015	0.079	0.02	1.22	0.254	0.269	0.497	1.49
7/29/92	0.016	0.016	0.01	0.01	0.002	0.003	0.003	0.643	0.332	0.095	0.109	0.873
8/11/92	0.009	0.009	ND	0.025	0.006	0.006	0.018	0.956	0.256	0.167	0.174	0.703
8/18/92	0.005	NTD	NTD	NTD	NTD	NTD	NTD	1.04	0.163	0.278	0.357	1.28
8/26/92	0.004	0.015	0.003	0.026	0.006	0.011	0.004	2.71	0.293	0.106	0.12	1.53
9/12/92	0.017	0.021	0.013	0.045	0.049	0.058	0.015	0.205	0.141	0.3	0.273	0.57
10/7/92	0.033	0.039	0.04	0.03	0.031	0.036	0.025	0.078	0.064	0.047	0.058	0.177
10/26/92	0.022	0.024	0.028	0.111	0.01	0.021	0.009	0.496	0.197	0.181	0.12	0.62
11/10/92	0.013	0.018	ND	ND	0.008	ND	0.001	0.192	0.123	0.217	0.106	0.416
12/14/92	ND	0.001	ND	ND	0.003	ND	0.001	0.456	0.126	0.097	0.081	0.328
1/25/93	0.001	NTD	0.001	NTD	0.005	NTD	0.014	0.706	0.043	0.105	0.11	0.444
3/22/93	0.013	0.006	0.008	0.012	0.026	0.02	0.011	1.19	0.238	0.151	0.211	1.518
5/17/93	0.003	0.01	ND	ND	0.013	ND	0.023	0.338	0.392	0.299	0.375	0.654
AVG	0.0337	0.0141	0.019	0.0407	0.0163	0.0221	0.0145	1.255333	0.330556	0.1738	0.1879	0.7573

ND-Not Done
NTD-Not Detected
AVG-Average

Table 11. Dissolved Oxygen Levels (mg/l) in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
6/8/92	2	6.5	7	7	6.6	6.9	5.9	6.3	5.4	3.2	2.4	5.5
6/17/92	6.2	5.8	6.5	6.6	6	6.7	6.1	2.3	4	3.8	2.5	2.8
6/29/92	6.9	7.2	7.9	9.4	7.9	8	7	3.8	7.5	4	7.3	3.6
7/8/92	5.6	5.5	6.5	6.6	7.1	7.8	6	5	5.9	3.7	4.2	1.5
7/15/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7/22/92	7	6.8	7.3	7.6	7.6	7.4	7.6	1.6	7.2	3.1	3	2
7/29/92	6.8	5.5	7.4	7.8	6.5	7	7.6	3	5.4	3.4	2.5	2.8
8/11/92	6.2	6.2	6.5	8	7.5	7.8	7.2	2.4	3.5	2.9	2.5	2
8/18/92	6.3	6.2	6.5	7.2	7	7.8	6.8	2.4	5.3	4	2.5	1.5
8/26/92	5.8	5.9	6.1	6.1	6.5	6.8	5.5	1.8	4.6	3.2	2.4	1.5
9/12/92	6.8	7.3	7.6	8	8.1	8	7.6	5.5	5.6	3.8	2.2	2.8
10/7/92	8.1	8	9	9.1	6.7	6.7	6.5	5.1	4.3	3	ND	1.5
10/26/92	8.3	7.3	8.2	8.9	6	8	6.2	3.4	5.8	3.6	3.7	2
11/10/92	6.1	5.2	ND	ND	6.4	ND	6.1	3.1	4.5	4.2	3.6	1.5
12/14/92	6.3	5.5	ND	ND	5.7	ND	6.5	1.8	5.6	4.7	4.5	3.7
1/25/93	6.2	6.3	6.3	7.2	6	7	7.3	2	5.7	3	3	2
3/22/93	6.9	6.3	7.3	7.8	8.2	ND	7.2	1.5	6.9	4.2	2.8	0.7
5/17/93	8	7.2	ND	ND	7	ND	10	5.5	4	2.5	3	0
AVG	6.4412	6.3941	7.15	7.6643	6.8706	7.3769	6.8882	3.323529	5.364706	3.547059	3.25625	2.2

ND-Not Done
AVG-Average

Table 12. pH Values in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
8/18/92	8.11	7.86	7.88	8.17	8.31	8.37	8.27	7.72	7.91	7.98	7.92	7.66
8/26/92	8.16	8.2	8.08	8.08	8.21	8.18	8.15	8.04	7.93	7.9	7.83	7.6
9/12/92	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10/7/92	8.01	7.82	8.08	8.08	7.46	8.02	8.06	7.86	7.2	7.46	7.62	7.75
10/26/92	7.26	6.83	7.79	7.54	8.17	8.13	8.13	7.72	7.77	7.54	7.81	7.87
11/10/92	8.08	8.11	ND	ND	8.15	ND	8.12	7.55	7.73	7.74	7.82	7.64
12/14/92	7.99	8.02	ND	ND	7.96	ND	8.07	7.93	7.82	7.85	7.88	7.83
1/25/93	8.24	8.17	8.25	8.26	8.25	8.25	8.21	7.56	7.86	7.99	7.84	7.94
3/22/93	8.21	8.2	8.23	8.31	8.27	8.31	8.19	7.75	7.56	7.85	7.84	7.94
5/17/93	8.09	8.08	ND	ND	8.11	ND	8.11	7.54	7.82	7.65	7.75	7.68
AVG	8.0167	7.9211	8.0517	8.0733	8.0989	8.21	8.1456	7.741111	7.733333	7.773333	7.812222	7.767778

ND-Not Done
AVG-Average

Table 13. Salinity (ppt) in Kuhio Beach and Queen's Surf Ocean Water Samples (Sites 1-7) and in Concurrently Taken Water Samples from Kapahulu Storm Drain System (Sites 8-12)

	OCEAN SAMPLE SITES							STORM DRAIN SITES				
DATE	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
6/8/92	34	34	32	34	34	34	34	0	15	18	12	16
6/17/92	ND	ND	32	ND	ND	ND	ND	0	18	27	24	20
6/29/92	30	32	34	34	34	32	34	2	2	24	24	18
7/8/92	32	32	32	32	32	32	32	0	22	10	6	5
7/15/92	34	34	34	33	32	32	34	0	14	24	20	14
7/22/92	34	34	34	33	34	32	34	0	12	5	3	10
7/29/92	34	34	34	34	32	34	34	4	15	18	23	15
8/11/92	34	34	34	34	34	34	34	0	14	20	18	14
8/18/92	ND	ND	33	33	ND	ND	ND	0	18	4	2	5
8/26/92	ND	34	34	33	34	34	32	0	14	20	22	18
9/12/92	32	32	32	32	32	32	32	0	18	14	10	10
10/7/92	< 34	32	32	34	34	34	34	0	8	5	5	4
10/26/92	33	34	34	34	34	33	34	0	15	18	20	10
11/10/92	34	34	ND	ND	34	ND	34	0	24	22	25	15
12/14/92	34	34	ND	ND	34	ND	34	10	20	25	28	16
1/25/93	34	33	33	33	32	32	34	0	12	16	22	8
3/22/93	34	34	34	34	32	33	34	0	8	14	10	4
5/17/93	34	34	ND	ND	30	ND	34	0	0	6	0	10
AVG	33.4	33.438	33.2	33.357	33	32.923	33.625	0.888889	13.83333	16.11111	15.22222	11.77778

ND-Not Done
AVG-Average

Table 14. Queen's Surf (Site 7) Morning (AM), Afternoon (PM) and Total Monthly Bacterial Geometric Means

	Enterococcus			E. coli			Fecal Coliform			C. perfringens			Bacillus spores		
Month	AM*	PM*	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL
Jun-92	0.673	ND	0.673	0	ND	0	0	ND	0	0	ND	0	0.587	ND	0.587
Jul-92	3.001	ND	3.001	0.649	ND	0.649	1.374	ND	1.374	0	ND	0	20.08	ND	20.08
Aug-92	2.694	ND	2.694	0.216	ND	0.216	1.387	ND	1.387	0	ND	0	3	ND	3
**9/12/92	2.4	ND	2.4	104	ND	104	92	ND	92	0.8	ND	0.8	39	ND	39
Oct-92	2.894	3.685	2.986	6.13	3.246	4.438	6.293	4.904	5.046	0.357	0.712	0.535	3.848	4.381	3.862
Nov-92	1.093	1.42	1.25	4.17	3.234	3.678	5.784	4.06	4.859	0.409	0.512	0.46	4.422	2.966	3.638
Dec-92	0.581	2.207	1.252	2.644	2.814	2.728	2.595	3.953	3.22	0.125	0.362	0.238	2.637	2.794	2.715
Jan-93	1.803	1.039	1.391	4.306	0.833	2.119	2.646	1.101	1.768	1.21	0.423	0.773	7	1.436	3.415
Feb-93	0.411	0.705	0.551	0.581	0.459	0.519	1.404	0.795	1.077	0.619	0.773	0.695	1.562	1.699	1.629
Mar-93	1.814	0.446	1.017	0.511	0.575	0.542	1.822	0.675	1.174	0.488	0.666	0.574	0.781	1.328	1.036
Apr-93	1.217	0.42	0.775	0.615	0.254	0.423	0.909	0.658	0.779	0.103	0	0.05	3.158	2.36	2.738
May-93	1.374	1.734	1.547	0.461	1.416	0.879	0.733	1.873	1.232	0.165	0.076	0.12	1.414	1.449	1.432
Jun-93	1.444	0.826	1.112	0.497	0.426	0.461	0.505	0.975	0.724	0.043	0.043	0.043	1.635	1.647	1.641
Jul-93	0.868	1.112	0.986	0.88	2.005	1.377	0.928	2.653	1.653	1.11	1.853	1.453	3.222	6.56	4.65
Aug-93	1.549	3.264	2.13	3.158	3.601	3.208	3.887	4.245	3.718	0.238	0.47	0.332	4.442	4.095	4.273
Sep-93	5.375	2.272	3.567	3.355	0.857	1.844	8.229	0.807	3.083	0.112	0.13	0.121	10.82	6.765	8.582
GM	1.435	1.313	1.382	1.674	1.325	1.47	2.257	1.816	1.999	0.366	0.447	0.389	2.892	2.892	2.892

*AM and PM geometric means include only samples on days when both AM and PM samples were collected

**Only one sample collected in Sept-92

ND-Not Done

GM-Overall Geometric Mean

Table 15. Kuhio Beach (Site 5) Morning (AM), Afternoon (PM) and Total Monthly Bacterial Geometric Means

	Enterococcus			E. coli			Fecal Coliform			C. perfringens			Bacillus spores		
Month	AM*	PM*	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL
Jun-92	0	ND	0	5.331	ND	5.331	7.087	ND	7.087	0	ND	0	8.491	ND	8.491
Jul-92	1.199	ND	1.199	2.409	ND	2.409	8.034	ND	8.034	0.158	ND	0.158	3.821	ND	3.821
Aug-92	1.136	ND	1.136	1.236	ND	1.236	4.851	ND	4.851	0.857	ND	0.857	7.271	ND	7.271
Sep-92	4.106	8.233	8.414	5.557	3.674	7.31	5.502	5.656	12.44	0.305	0.213	0.388	3.812	6.077	6.385
Oct-92	25.7	18.15	18.49	39.44	33.15	34.5	83.71	54.44	57.5	3.942	2.649	2.988	13.21	12.56	10.92
Nov-92	25.04	20.42	22.62	29.33	50.54	38.54	53.04	77.23	64.02	0.849	1.163	1	7.675	9.577	8.579
Dec-92	14.12	30.09	20.68	19.56	42.54	28.92	34.43	66.09	47.75	0.43	1.979	1.064	9.796	7.816	8.756
Jan-93	44.38	22.17	31.42	33.36	23.31	27.9	76.16	35.48	52.05	1.907	2.867	2.353	7.914	4.228	5.826
Feb-93	41.53	21.27	29.77	68.51	49.63	58.33	98.36	34.57	58.45	2.58	0.806	1.543	5.036	3.275	4.08
Mar-93	6.552	3.041	4.524	16.79	5.102	9.419	32.42	7.225	15.58	0.942	0.607	0.766	4.659	2.613	3.521
Apr-93	9.6	3.699	6.057	11.48	5.396	7.934	16.91	4.319	8.76	0	0.05	0.025	3.673	5.799	4.637
May-93	4.766	3.295	3.976	4.696	3.594	3.696	6.163	3.117	4.431	0.446	0.35	0.397	3.561	3.31	3.434
Jun-93	2.6	3.063	2.824	1.195	1.976	1.556	2.685	2.652	2.668	0.088	0.071	0.079	1.585	0.919	1.227
Jul-93	1.735	1.45	1.588	1.84	1.78	1.81	2.639	2.418	2.527	0.672	0.593	0.632	3.442	4.836	4.091
Aug-93	1.832	1.934	1.824	4.047	1.727	2.505	5.532	2.669	3.863	0.113	0.091	0.097	5.612	6.464	6.007
Sep-93	1.913	2.931	2.384	6.497	3.173	4.593	7.252	4.639	5.821	0.153	0.303	0.226	4.603	19.57	9.736
GM	8.35	6.71	7.058	11.07	8.683	9.443	17.6	10.96	13.9	0.719	0.689	0.68	4.968	5.105	5.182

*AM and PM geometric means include only samples on days when both AM and PM samples were collected
ND-Not Done
GM-Overall Geometric Mean

Table 16. Kuhio Beach (Site 1) Morning (AM), Afternoon (PM) and Total Monthly Bacterial Geometric Means

	Enterococcus			E. coli			Fecal Coliform			C. perfringens			Bacillus spores		
Month	AM*	PM*	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL
Jun-92	0.216	ND	0.216	0.673	ND	0.673	5.136	ND	5.136	0.216	ND	0.216	2.733	ND	2.733
Jul-92	0.798	ND	0.798	0.936	ND	0.936	7.11	ND	7.11	0	ND	0	34.17	ND	34.17
Aug-92	1.918	ND	1.918	4.512	ND	4.512	11.12	ND	11.12	1.306	ND	1.306	3.656	ND	3.656
Sep-92	3.29	6.358	6.469	2.845	3.617	6.659	3.308	5.97	9.41	0.158	0.088	0.225	4.39	7.357	6.015
Oct-92	26.04	17.05	20.54	30.75	27.31	27.98	78.51	44.43	55.89	3.061	3.114	2.844	16.4	9.152	12.86
Nov-92	22.18	15.95	18.82	67.29	27.33	42.98	64.16	70.22	67.12	1.021	0.812	0.914	17.56	12.39	14.76
Dec-92	8.003	7.48	7.738	18.54	16.09	17.27	27.64	19.79	23.4	0.405	0.854	0.614	8.037	11.24	9.519
Jan-93	4.636	2.277	3.3	10.5	8.873	9.654	12.86	5.497	8.488	0.799	1.946	1.302	3.228	3.13	3.179
Feb-93	3.323	2.47	2.873	8.772	5.063	6.697	15.61	10.86	13.04	0.359	0.701	0.52	3.239	1.312	2.131
Mar-93	0.989	1.521	1.178	4.774	1.685	2.833	7.911	2.464	4.503	0.195	0.464	0.354	2.297	1.701	1.943
Apr-93	0.82	1.781	1.25	3.195	0.911	1.831	3.864	1.968	2.799	0.103	0.083	0.093	2.046	1.275	1.632
May-93	1.793	2.465	2.111	2.219	5.082	3.425	8.989	8.592	8.789	0.583	0.076	0.305	2.253	1.783	2.009
Jun-93	1.563	6.592	3.411	1.674	4.208	2.732	1.689	5.228	3.092	0	0	0	1.213	3.822	2.267
Jul-93	2.941	5.456	4.044	4.132	4.242	4.186	6.361	6.825	6.589	1.053	0.426	0.711	2.429	3.671	3.002
Aug-93	3.219	8.039	5.491	6.981	7.22	7.167	8.56	8.097	8.311	0.28	0.48	0.393	8.208	12.53	10.07
Sep-93	3.823	4.46	4.132	4.139	3.457	3.786	5.497	11.68	8.077	0.08	0.395	0.227	8.965	32.93	17.39
GM	3.922	4.989	4.907	7.326	5.976	6.564	10.63	9.338	9.203	0.486	0.568	0.52	4.578	4.971	4.93

*AM and PM geometric means only includes samples days when both AM and PM samples were collected
ND-Not Done
GM-Overall Geometric Mean

Table 17. Kuhio Beach (Site 2) Morning (AM), Afternoon (PM) and Total Monthly Bacterial Geometric Means

	Enterococcus			E. coli			Fecal Coliform			C. perfringens			Bacillus spores		
Month	AM*	PM*	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL	AM	PM	TOTAL
Jun-92	0.673	ND	0.673	2.849	ND	2.849	4.688	ND	4.688	0	ND	0	3.121	ND	3.121
Jul-92	0.798	ND	0.798	8.617	ND	8.617	11.86	ND	11.86	0	ND	0	8.451	ND	8.451
Aug-92	5.103	ND	5.103	2.017	ND	2.017	5.62	ND	5.62	0.732	ND	0.732	19.61	ND	19.61
Sep-92	4.262	15.84	9.479	8.76	6.211	13.22	11.19	14.82	21.2	0.213	0.386	0.521	9.105	15.7	14
Oct-92	17.47	9.234	13.03	25.69	9.018	15.19	41.8	12	22.47	2.956	1.798	2.158	14.77	21.48	16.21
Nov-92	7.667	11.66	9.475	17.4	7.171	11.26	27.65	14.89	20.33	0.915	0.473	0.68	9.537	6.842	8.09
Dec-92	5.682	3.13	4.253	11.35	6.808	8.225	18.02	12.18	14.83	0.326	0.284	0.344	5.172	11.21	7.68
Jan-93	2.714	2.095	2.39	9.294	2.818	5.269	20.28	3.66	8.958	0.112	0.479	0.282	2.236	2.401	2.317
Feb-93	3.08	2.554	2.808	5.433	1.918	3.332	7.507	3.796	5.387	0.151	0.369	0.255	1.503	0.742	1.088
Mar-93	2.887	1.215	1.934	5.253	1.15	2.667	6.741	1.568	3.459	1.083	0.329	0.664	2.83	2.159	2.478
Apr-93	1.911	0.598	1.157	2.491	0.736	1.462	3.649	0.945	2.007	0	0.216	0.103	2.154	1.922	2.035
May-93	1.864	1.269	1.549	4.642	1.175	2.503	4.215	2.65	3.363	0.076	0	0.037	2.105	2.191	2.148
Jun-93	0.648	2.811	1.506	2.421	1.691	2.034	3.361	2.899	3.123	0.043	0.043	0.043	2.916	8.442	5.081
Jul-93	3.243	2.744	2.986	3.092	2.255	2.649	3.723	3.341	3.528	0.927	0.93	0.929	5.221	7.774	6.388
Aug-93	4.76	5.248	4.78	5.39	3.803	4.423	10.56	5.994	7.993	0.38	0.118	0.23	9.223	29.06	16.12
Sep-93	3.238	2.645	2.931	1.453	1.471	1.462	2.813	6.19	4.236	0	0.067	0.033	6.97	9.613	8.197
GM	3.528	3.292	3.50	5.953	2.775	4.448	8.752	4.773	7.022	0.451	0.368	0.419	4.537	6.123	5.602

*AM and PM geometric means includes only samples on days when both AM and PM samples were collected
ND-Not Done
GM-Overall Geometric Mean

Table 18. Levels of Presumptive Staphylococcus in Ocean Water Samples from Kuhio Beach (Sites 1,2,5) and Queen's Surf (Site 7) in Morning (AM) and Afternoon (PM)

	SITE 1		SITE 2		SITE 5		SITE 7	
DATE	AM	PM	AM	PM	AM	PM	AM	PM
11/2/92	1440	323	1080	36	72	404	116	8
11/3/92	200	284	220	64	40	84	12	4
11/4/92	600	1040	920	300	20	20	72	80
3/8/93	1080	760	40	76	36	48	16	104
3/9/93	204	284	0	12	20	16	52	8
3/10/93	1840	204	840	512	80	8	0	100
GM	642.52	401.84	139.18	83.795	38.768	40.911	20.546	25.52

GM-Geometric Mean

Table 19. Levels of Indicator Bacteria (CFU/100g) in Dry and Wet Sand from Kuhio Beach (Sites 1,2) and Queen's Surf Beach (Site 7)

		EN		EC		FC		CP		BS	
SITE	DATE	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET
1	11/17/92	11200	560	8800	440	10400	600	160	40	19200	2320
	2/16/93	2560	0	3000	0	17600	0	0	40	80	400
	3/23/93	80	8	0	8	160	0	88	32	14700	0
	GM	1323	16.16	296.8	14.83	3088	7.439	23.29	37.14	2837	96.64
2	11/17/92	20000	248	0	96	1600	160	280	80	8560	1160
	2/16/93	320	0	160	1	160	0	80	40	240	80
	3/23/93	7760	16	0	8	0	8	16	104	568	16
	GM	3679	15.18	4.44	11.04	62.64	10.32	71.87	69.39	1054	115.9
7	11/17/92	0	0	0	0	0	8	8	0	48	136
	2/16/93	0	0	0	0	0	0	0	0	160	0
	3/23/93	32	0	8	0	0	0	40	0	88	8
	GM	2.208	0	1.08	0	0	1.08	6.173	0	87.88	9.723

EN-enterococci EC-E. coli GM-Geometric Mean
FC-fecal coliform CP-C. perfringens
BS-Bacillus spores NG-No Growth

Table 20. Correlations (R) Between Levels of Presumptive Staphylococcus in Ocean Water at Sites 1, 2 and 7 and Numbers of Swimmers

SITE	TIME	R	p VALUE
1	AM	0.231	0.660
	PM	0.823	0.044
2	AM	0.921	0.009
	PM	0.656	0.157
7	AM	0.721	0.106
	PM	0.238	0.649

Appendix 1. Kuhio Beach (Site 1) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
6/8/92	0.8	ND	0	ND	10	ND	0.8	ND	0	ND	NTD	ND	ND	ND	34	ND
6/17/92	0	ND	0.8	ND	3.2	ND	0	ND	3	ND	0.033	ND	ND	ND	ND	ND
6/29/92	0	ND	1.6	ND	4	ND	0	ND	12	ND	0.006	ND	ND	ND	30	ND
*7/6/92	0	ND	0.8	ND	3.2	ND	0	ND	137	ND	0.082	ND	ND	ND	32	ND
7/15/92	4.8	ND	0	ND	6.4	ND	0	ND	263	ND	0.149	ND	8.26	ND	34	ND
7/22/92	0.8	ND	2	ND	23	ND	0	ND	6	ND	0.167	ND	ND	ND	34	ND
*7/29/92	0	ND	1.6	ND	4.8	ND	0	ND	5	ND	0.016	ND	ND	ND	34	ND
8/11/92	0	ND	1.6	ND	8	ND	0	ND	1	ND	0.009	ND	ND	ND	34	ND
8/18/92	2.4	ND	4.8	ND	18.7	ND	14.7	ND	0	ND	NTD	ND	8.11	ND	ND	ND
8/26/92	7.2	ND	33	ND	28	ND	0	ND	4	ND	0.004	ND	8.20	ND	34	ND
8/31/92	1.6	ND	0.8	ND	3.2	ND	0.8	ND	46	ND	0.053	ND	7.88	ND	32	ND
9/1/92	1.6	ND	1.6	ND	3.2	ND	0.8	ND	0	ND	0.009	ND	7.75	ND	33	ND
*9/3/92	8.8	ND	6.4	ND	7.2	ND	0	ND	3	ND	0.046	ND	7.98	ND	32	ND
*9/4/92	12.8	ND	1	ND	16	ND	0	ND	2	ND	0.016	ND	2.71	ND	33	ND
9/5/92	7.2	ND	8	ND	8	ND	0	ND	17	ND	NTD	ND	7.99	ND	32	ND
9/7/92	0.8	ND	0.8	ND	1.6	ND	1.6	ND	2	ND	0.015	ND	8.09	ND	32	ND
9/8/92	1.6	ND	42	ND	35	ND	0.8	ND	ND	ND	0.002	ND	8.06	ND	35	ND
9/9/92	1.6	13.6	0.8	4	5.6	7.2	0	0	1	5	NTD	0.015	7.92	8.28	35	35
9/10/92	0	124	1.6	152	0.8	256	0	0	0	53	ND	ND	ND	ND	35	35
*9/12/92	3.2	ND	148	ND	292	ND	0	ND	26	ND	0.017	ND	8.07	ND	ND	ND
*9/14/92	22	ND	48	ND	23	ND	0.8	ND	10	ND	0.028	ND	7.86	ND	35	ND
9/16/92	168	ND	284	ND	380	ND	0	ND	6	ND	NTD	ND	6.53	ND	36	ND
9/18/92	232	ND	176	ND	324	ND	0.8	ND	61	ND	NTD	ND	8.03	ND	ND	ND
9/21/92	1.6	50	3.2	16.8	5.6	23.2	0	0.8	11	33	0.013	0.01	7.95	8.02	ND	34
9/23/92	16	0.8	8.8	1.6	4	7.2	0.8	0	21	8	NTD	0	8.17	8.18	32	33
9/25/92	3.2	2.4	4	0.8	0.8	6.4	0	0	5	4	NTD	0.005	7.99	8.21	32	32
9/28/92	9.6	0	9.6	0.8	4.8	0.8	0	0	24	7	0.001	0.004	7.96	8.11	34	34
9/29/92	5.6	1.6	0.8	0	4	0	0.8	0	8	2	0.006	0.001	8.02	8.16	34	34
9/30/92	2.4	4.8	1.6	0.8	4.8	0	0	0	0	1	NTD	0.003	7.66	8.15	34	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 1. Kuhio Beach (Site 1) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
10/5/92	0	1.6	2.4	0	12	3.2	0	0	10	64	0.005	0.003	7.50	8.16	34	32
10/6/92	ND	12	ND	12.8	ND	16	ND	0	ND	34	ND	0.004	ND	8.22	ND	32
*10/7/92	0.8	14.4	0	18.4	3.2	23	0	0	6	27	0.003	0.029	8.01	8.07	34	34
*10/12/92	ND	ND	832	60	1920	172	31	16	56	0	0.005	0.012	7.84	8.05	30	32
*10/13/92	6320	62	2880	108	3360	312	40	8.8	96	16	0.03	0.025	7.91	7.78	28	30
*10/14/92	456	40	276	88	2000	248	11.2	52	5	3	0.018	0.029	7.95	7.96	32	32
10/19/92	160	144	18.4	90	50	131	52	3.2	97	3	0.001	0.006	7.97	7.80	33	33
10/20/92	9.6	11.2	19.2	8	162	5.6	0.8	2.4	12	23	0.006	0.006	8.09	8.16	33	33
*10/21/92	6.4	65	30	112	24	224	0.8	2.4	17	22	0.029	0.011	7.83	7.78	34	34
*10/26/92	60	36	42	23	82	12	0.8	6.4	5	11	0.022	0.007	7.26	8.24	33	34
10/27/92	0.8	0	0	1.6	2.4	3.2	0	0	6	2	0	0.003	8.24	8.26	30	30
10/28/92	28	7.2	27	140	18.4	116	0	0.8	17	11	0.02	0.003	8.01	8.21	34	33
*11/2/92	21	25	336	38	524	84	0.8	1.6	30	28	0.004	0.008	8.16	8.26	34	34
11/3/92	128	12.8	312	15.2	28	1040	ND	ND	32	12	0.007	0.005	8.18	8.08	33	33
11/4/92	44	136	244	104	276	432	0	1.6	21	20	0.002	0.006	8.06	8.21	33	34
*11/9/92	24	92	12	92	24	32	8	0.8	16	18	0.01	0.018	8.10	8.23	34	33
*11/10/92	27	6.4	56	8	47	6.4	0	0	18	13	0.013	0.009	8.08	8.30	34	34
*11/11/92	30	16.8	204	15.2	532	232	4.8	1.6	25	20	0.021	0.076	8.10	7.89	33	34
11/16/92	52	5.6	52	9.6	76	7.2	3.2	1.6	14	12	0.004	0.016	8.00	8.18	34	34
*11/17/92	2.4	19.2	11.2	45	10.4	73	0.8	0	7	6	0.011	0.057	6.91	6.38	33	33
*11/18/92	15.2	1.6	22	78	35	77	0	0.8	8	10	0.004	0.007	8.07	8.21	34	33
11/23/92	7.2	22	25	11.2	44	11.2	0	0	16	10	0.008	0.01	8.17	8.12	34	34
11/24/92	5.6	19.2	1168	15.2	42	7.2	0.8	0.8	22	16	0.005	0.003	8.11	8.21	34	34
*11/30/92	64	4.8	4.8	38	35	1648	0.8	1.6	20	3	0.004	0.002	7.64	8.08	34	34
*12/1/92	3.2	2.4	35	4.8	36	7.2	0	0	6	226	0.007	0.008	7.59	6.60	34	34
*12/2/92	3.2	1.6	73	14.4	63	63	0	0.8	8	12	0.002	0.002	8.10	8.22	34	34
*12/7/92	6.4	21	29	15.2	59	8.8	0	0.8	10	5	0.009	0.01	8.11	8.23	34	34
12/8/92	10.4	13.6	6.4	20	29	7.2	0	0	1	2	0.01	0.004	7.58	7.92	33	34
*12/9/92	52	3.2	16	24	31	10.4	2.4	1.6	12	4	0.012	0.005	8.10	8.25	34	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 1. Kuhio Beach (Site 1) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
12/14/92	5.6	8	32	8.8	52	20	0	1.6	19	45	0	0.006	7.99	8.13	34	34
12/15/92	4	47	4.8	152	6.4	236	0	0.8	15	3	0.017	0.004	7.85	8.14	34	34
*12/16/92	13.3	8	5.6	22	15.2	25	0	0.8	13	9	0.002	0.019	8.00	8.22	35	34
*12/21/92	4.8	11.2	28	15.2	17.6	26	1.6	1.6	4	30	0.008	0.001	8.10	8.13	34	34
*12/22/92	15.2	2.4	21	4	22	8	2.4	1.6	8	4	0.029	0.014	6.82	7.57	32	34
1/11/93	1.6	0.8	8	0.8	5.6	2.4	0	0	4	4	0.018	0.022	8.17	8.24	32	33
1/12/93	0.8	0.8	4	9.6	7.2	0	0.8	0	3	6	0.003	0.004	8.28	8.25	34	34
1/13/93	2.4	0	4.8	2.4	5.6	2.4	0	0.8	4	2	0.002	0.003	8.28	8.29	34	33
1/18/93	4.8	13.6	32	120	31	96	1.6	2.4	9	1	0.007	0.007	8.31	8.31	34	34
1/19/93	1.6	4.8	10.4	10.4	12	10.4	0	2.4	1	3	0.004	0.006	8.31	8.39	34	34
1/20/93	14.4	0.8	14.4	7.2	14.4	12.8	8	13.6	5	1	0.005	0.004	8.32	8.37	34	34
1/25/93	5.6	0.8	29	8	14.4	4	1.6	2.4	2	0	0.001	0.014	8.24	8.29	34	33
1/26/93	23	4	42	4	29	8	0	8	1	7	0.001	0.002	8.17	8.31	34	33
1/27/93	8.8	8.8	0.8	26	16.8	1.6	0.8	0.8	5	25	NTD	0.017	8.15	8.23	33	33
2/1/93	0	3.2	5.6	2.4	15.2	6.4	0	1.6	462	1	0.002	NTD	ND	ND	34	34
2/2/93	4.8	5.6	11.2	8.8	144	100	0	0.8	0	1	0.001	NTD	8.30	8.35	34	34
2/3/93	5.6	8	20	14.4	15.2	21	0.8	0	0	0	0.007	0.01	8.35	8.37	34	34
*2/8/93	7.2	8	9.6	16.8	7.2	21	0	1.6	2	6	0.002	0.01	8.37	8.42	34	34
*2/9/93	23	2.4	236	0.8	352	17.6	8	3.2	12	4	NTD	NTD	8.26	8.24	34	34
2/10/93	0	0	6.4	27	10.4	42	0	0	10	3	0.003	0.008	8.09	8.14	34	34
2/16/93	0.8	1.6	8.8	15.2	8.8	12	0	1.6	4	5	0.011	0.004	8.20	8.30	34	34
2/17/93	6.4	1.6	2.4	12	10.4	2.4	0.8	1.6	0	0	0.001	0.001	8.20	8.24	34	34
*2/22/93	3.2	4	3.2	0	4	2.4	0	0	3	2	0.005	0.007	8.18	8.21	34	34
*2/23/93	1.6	2.4	0.8	0	1.6	1.6	0	0	1	0	0.011	0.003	8.31	8.40	34	34
2/24/93	8	0	8.8	3.2	13.6	4.8	0	0	0	0	0.008	0.004	8.33	8.39	34	34
3/1/93	2.4	60	1.6	0.8	2.4	0.8	0	2.4	1	2	0.003	0.003	8.1	8.15	34	34
*3/2/93	12	0.8	48	8.8	72	7.2	0	0.8	2	0	0.001	0.002	8.22	8.32	34	34
3/3/93	10.4	1.6	10.4	1.6	7.2	4	0	0	3	5	0.002	0.01	8.24	8.3	34	34
3/8/93	2.4	0.8	10.4	5.6	15.2	42	1.6	0	11	4	0.006	0.003	8.22	8.25	34	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 1. Kuhio Beach (Site 1) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*3/9/93	0	0	8.8	4	12.8	6.4	0	0	0	1	0.002	0.01	8.24	8.33	34	34
*3/10/93	0	0.8	0.8	1.6	8	6.4	0	0.8	3	9	0.014	0.012	8.25	8.28	34	33
*3/15/93	ND	0	ND	0.8	ND	3.2	ND	1.6	ND	1	ND	0.01	ND	8.29	ND	34
*3/16/93	0	0	0	0	2.4	0.8	0	0	17	16	0.006	0.01	8.23	8.31	34	33
3/17/93	0	16	4	0.8	8.8	3.2	0	0.8	2	0	0.007	0.011	8.29	8.32	34	34
3/22/93	0	0	0.8	0	0	0	0	0	2	0	0.013	0.013	8.21	8.28	34	34
3/23/93	0	0	4.8	0.8	4	0.8	1.6	0	2	3	0.017	0.019	8.19	8.26	34	34
3/24/93	0	7.2	0	12	14.4	0.8	0	0.8	3	2	0.007	0.01	8.28	8.3	34	34
3/29/93	2.4	0	72	0.8	45	1.6	0.8	0.8	3	2	0.002	0.018	8.28	8.33	34	34
3/30/93	0	0.8	3.2	0	3.2	0.8	0	0.8	1	0	0.007	0.013	8.06	8.33	34	34
3/31/93	1.6	0.8	8.8	2.4	8.8	0.8	0	0.8	0	0	0.007	0.008	8.26	8.29	34	34
*4/5/93	0	7.2	0	1.6	0	3.2	0	0	1	1	0.019	0.008	8.25	8.32	34	34
*4/6/93	0	0	12	0.8	29	2.4	0	0	2	1	0.009	0.012	8.04	8.21	34	34
4/7/93	0	1.6	1.6	0	0.8	4	0.8	1.6	3	4	0.01	0.007	8.25	8.05	34	34
*4/12/93	0	1.6	7.2	0	12.8	0.8	0	0	1	4	0.059	0.019	8.16	8.27	34	34
*4/13/93	0.8	0	6.4	0.8	4	0	0	0	0	0	0.004	0.022	8.26	8.36	34	34
4/14/93	0.8	2.4	0	0	0	2.4	0	0	3	3	0.005	0.01	8.18	8.31	34	34
*4/19/93	2.4	23.2	3.2	4.8	12	8.8	0.8	0	1	2	0.013	0.017	8.27	8.3	34	34
*4/20/93	3.2	0	144	0.8	244	1.6	0	0	12	1	0.001	0.002	8.28	8.37	34	34
*4/21/93	0.8	12.8	5.6	26	7.2	41	0	0	3	0	0.066	0.005	8.13	8.3	32	34
4/26/93	2.4	2.4	2.6	0	0	0	0	0	7	3	0.018	0.015	8.17	8.24	34	33
*4/27/93	1.6	0	0	0	0	0	0	0	1	1	0.01	0.008	8.22	8.27	34	34
4/28/93	0.8	0	0	0	0.8	0	0	0	1	0	0.011	0.012	8.12	7.8	34	34
5/3/93	1.6	1.6	0.8	4.8	12	9.6	1.6	0	0	4	0.011	0.016	8.22	8.3	35	34
5/4/93	1.6	3.2	4	11.2	3.2	11.2	0.8	0	1	2	0.048	0.011	8.02	6.86	34	34
5/5/93	1.6	0.8	1.6	15.2	1.6	16	0.8	0	9	2	0.628	0.045	7.88	8.34	35	34
5/10/93	0.8	1.6	0	2.4	0.8	1.6	0.8	0.8	0	0	0.007	0.013	8.24	8.34	34	35
5/11/93	0	1.6	0	15.2	0	27	0	0	0	3	0.007	0.013	8.24	8.32	34	34
*5/12/93	8	9.6	4	10.4	1520	16.8	0	0	2	1	0.018	0.005	8.1	8.15	34	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 1. Kuhio Beach (Site 1) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*5/17/93	0	7.2	2.4	0	4	2.4	0	0	10	1	0.003	0.014	8.09	7.94	34	34
*5/18/93	12	0.8	28	1.6	50	6.4	1.6	0	18	4	0.009	0.016	8.08	8.21	34	34
6/1/93	0.8	4	0	0.8	0	2.4	0	0	0	0	1.36	0.009	8.1	8.21	34	33
6/2/93	2.4	0	2.4	1.6	0	3.2	0	0	3	2	0.015	NTD	7.51	8.12	34	33
*6/7/93	0	2.4	0	0	0	0	0	0	0	1	0.014	0.004	8.1	8.19	34	34
*6/8/93	1.6	0	0.8	0.8	0	0	0	0	1	7	0.003	0.002	8.14	8.19	34	34
*6/9/93	0.8	*2048	3.2	0.8	1.6	37	0	0	3	2	0.005	0.786	8.11	8.06	34	34
6/14/93	0	4	1.6	43	4	53	0	0	0	3	0.007	0.013	8.14	8.15	34	34
6/15/93	0.8	18.4	8.8	1.6	13.6	7.2	0	0	1	27	NTD	0.004	8.01	8.13	34	34
6/16/93	0	1.6	0	6.4	0	11.2	0	0	2	1	0.01	NTD	8.09	8.15	32	34
*6/21/93	0.8	15.2	0.8	25	23	0	0	0	1	3	NTD	NTD	8.21	8.22	34	34
6/22/93	1.6	3.2	0	0	0	0	0	0	0	2	0.057	0.041	8.15	8.18	34	33
6/23/93	12	0	1.6	0.8	0.8	0.8	0	0	1	0	0.01	0.006	8.11	8.14	34	34
6/28/93	0.8	83	26	200	17.6	276	0	0	1	11	0.007	0.01	8.06	8.09	34	34
6/29/93	10.4	15.2	2.4	14.4	1.6	11.2	0	0	10	8	0.062	0.03	8.04	8.12	34	33
*6/30/93	7.2	1.6	2.4	4.8	1.6	6.4	0	0	3	87	0.01	NTD	8.08	8.11	34	34
7/5/93	1.6	0.8	0	1.6	0.8	1.6	0	0	9	1	0.004	0.004	8.25	8.31	33	34
7/6/93	0.8	2.4	0	3.2	0.8	4.8	5.6	9.6	2	8	0.006	0.001	8.02	8.24	34	34
7/7/93	0.8	0.8	3.2	0	3.2	1.6	8.8	0	0	4	0.046	0.018	8.2	8.24	34	34
7/12/93	0.8	0.8	6.4	2.4	4.8	3.2	0.8	0	0	0	0.01	0.003	8.11	8.17	34	34
7/13/93	0.8	14.4	16.8	12.8	11.2	18.4	0	0	0	1	0.052	NTD	7.73	8.17	34	33
7/14/93	1.6	4	8.8	5.6	11.2	4	0	0.8	0	10	0.006	0.02	8.09	8.16	33	34
*7/19/93	3.2	1.6	2.4	4.8	5.6	1.6	0	0	2	5	NTD	0.007	8.15	8.26	34	33
*7/20/93	1.6	1.6	0	0	2.4	0	0	0	2	2	0.012	0.019	8.17	8.16	34	34
7/21/93	2.4	3.2	0	0	0.8	4	0	0	11	5	0.002	0.01	8.14	8.15	34	33
*7/26/93	53	236	81	88	160	420	12	0	33	5	NTD	0.001	8.22	8.32	34	34
*7/27/93	24	78	42	46	44	76	0.8	1.6	6	17	NTD	0.001	8.18	8.28	34	34
8/2/93	3.2	0.8	1.6	4	0.8	2.4	0	0	4	2	0.002	NTD	8.19	8.27	34	34
8/3/93	4	1.6	24	1.6	34	3.2	0	0.8	6	8	0.005	0.003	8.19	8.2	34	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 1. Kuhio Beach (Site 1) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*8/4/93	2.4	4	4	2.4	5.6	2.4	0	0	2	7	0.008	0.016	8.09	8.19	34	34
8/9/93	4.8	120	4	4	8.8	9.6	0	0	15	15	0.048	0.008	8.13	8.16	34	34
*8/10/93	4	30	62	12	52	4.8	0	0.8	8	47	0.012	NTD	8.1	8.15	34	34
8/11/93	14.4	12	16.8	10.4	20	4	0.8	1.6	12	140	0.014	NTD	8.1	8.14	33	34
*8/16/93	0	71	4	8	6.4	8.8	0	0	49	47	NTD	0.01	8.05	8.05	33	32
*8/17/93	4	3.2	29	21	29	20	0.8	2.4	17	10	0.002	0.004	8.13	8.16	34	34
8/18/93	0.8	0.8	0	0	0	2.4	1.6	0	4	2	0.003	0.001	8.11	8.14	35	37
*8/23/93	13.6	9.6	16	50	16.8	68	0.8	0	14	14	0.002	0.002	8.01	8.08	34	34
8/24/93	0.8	4	0.8	34	2.4	48	0	1.6	1	4	0.003	0.006	8.12	8.09	34	34
8/25/93	ND	18.4	ND	8.8	ND	8	ND	0.8	ND	13	ND	0.011	ND	8.14	ND	34
*9/13/93	0.8	1.6	4	7.2	0.8	40	0	0	1	7	0.006	0.009	8.14	8.25	34	34
*9/14/93	1.6	3.2	2.4	6.4	0	8.8	0	0	1	3	0.024	0.016	8.27	8.18	34	34
*9/15/93	0.8	0	0	0.8	8	0.8	0	0	1	263	0.005	0.013	8.11	8.19	35	34
*9/20/93	8	3.2	4	0.8	38	0.8	0	0	7	10	0.001	0.003	8.09	8.16	34	34
*9/21/93	4.8	0.8	14.4	14.4	22	44	0	1	6	4	0.01	NTD	8.11	8.14	34	34
9/22/93	49	20	23	3.2	29	660	1	4	280	280	0.008	0.08	7.85	8.18	34	34
9/27/93	0.8	9.6	0.8	0	0.8	3.2	0	1	4	108	0.015	0.004	8.16	8.18	34	34
9/28/93	6.4	42	12	20	13.6	28	0	0	384	92	0.01	0.009	8.03	8.03	35	34
9/29/93	ND	ND	2.4	1.6	0.8	0.8	0	0	3	44	0.01	0.008	8.05	8.17	37	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 2. Kuhio Beach (Site 2) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
6/8/92	0.8	ND	0	ND	2.4	ND	0	ND	1	ND	0.006	ND	ND	ND	34	ND
6/17/92	1.6	ND	10.4	ND	7.2	ND	0	ND	6	ND	NTD	ND	ND	ND	ND	ND
6/29/92	0	ND	4	ND	5.6	ND	0	ND	4	ND	0.011	ND	ND	ND	32	ND
*7/8/92	4.8	ND	4.8	ND	8.8	ND	0	ND	4	ND	0.019	ND	ND	ND	32	ND
7/15/92	0	ND	2.4	ND	2.4	ND	0	ND	56	ND	0.002	ND	8.24	ND	34	ND
7/22/92	0	ND	240	ND	820	ND	0	ND	3	ND	0.057	ND	8.12	ND	34	ND
*7/29/92	0.8	ND	0.8	ND	0	ND	0	ND	6	ND	0.015	ND	8.2	ND	34	ND
8/11/92	4	ND	0	ND	0.8	ND	0	ND	3	ND	0.009	ND	ND	ND	34	ND
8/18/92	6.7	ND	4.8	ND	6.7	ND	8	ND	1	ND	NTD	ND	7.86	ND	33	ND
8/26/92	9.6	ND	2.4	ND	8	ND	0	ND	109	ND	0.015	ND	8.2	ND	34	ND
8/31/92	2.4	ND	3.2	ND	14.4	ND	0	ND	204	ND	0.015	ND	7.77	ND	32	ND
9/1/92	0	ND	0	ND	1.6	ND	1.6	ND	8	ND	0.007	ND	7.92	ND	33	ND
*9/3/92	51	ND	128	ND	108	ND	10.4	ND	21	ND	0.005	ND	7.98	ND	32	ND
*9/4/92	64	ND	67	ND	324	ND	0.8	ND	68	ND	NTD	ND	8.09	ND	33	ND
9/5/92	2.4	ND	2.4	ND	8.8	ND	0	ND	19	ND	0.082	ND	8.07	ND	33	ND
9/7/92	0	ND	2.4	ND	2.4	ND	1.6	ND	3	ND	0.002	ND	7.92	ND	32	ND
9/8/92	15.2	ND	29	ND	21.6	ND	0	ND	ND	ND	0.006	ND	8.15	ND	36	ND
9/9/92	0	29	1.6	12	2.4	26	0	0	2	14	0.052	0.074	8.08	8.21	35	35
9/10/92	6.4	168	0	27	4	41	0.8	0.8	10	30	ND	ND	ND	ND	ND	ND
*9/12/92	6.4	ND	74	ND	64	ND	2.4	ND	33	ND	0.021	ND	8.14	ND	ND	ND
*9/14/92	4.4	ND	40	ND	44	ND	0	ND	15	ND	0.023	ND	7.98	ND	35	ND
9/16/92	79	ND	292	ND	360	ND	0	ND	24	ND	0.006	ND	8.01	ND	35	ND
9/18/92	148	ND	572	ND	720	ND	0.8	ND	25	ND	0.004	ND	8.00	ND	ND	ND
9/21/92	2.4	19.2	26	35	44	37	0	0.8	14	50	0.006	0.003	8.04	8.06	ND	34
9/23/92	1.6	51	43	0.8	6.4	92	0	0	3	75	NTD	0.004	8.10	8.18	32	32
9/25/92	0.8	13.6	26	7.2	39	12	0	3.2	8	11	NTD	0.013	8.08	8.21	32	32
9/28/92	26	0.8	13.6	4	17.6	4	1.6	0	60	9	NTD	0.002	8.03	8.11	34	34
9/29/92	6.4	1.6	1.6	0.8	2.4	1.6	0	0	19	6	0.006	0.017	8.05	8.12	34	34
9/30/92	24	16.8	25	3.2	33	4.8	0	0	4	3	NTD	0.002	8.03	8.11	32	34
10/5/92	26	23	98	33	65	22	0.8	0	180	352	0.035	0.003	7.87	8.14	32	32

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 2. Kuhio Beach (Site 2) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
10/6/92	ND	20	ND	12	ND	20	ND	0	ND	40	ND	NTD	ND	8.26	ND	32
*10/7/92	13.6	4	2.4	0.8	11.2	6.4	0	0	8	41	0.039	0.018	7.82	8.41	32	34
*10/12/92	ND	ND	296	11.2	628	16.8	5.6	0	25	14	0.002	0.012	7.94	7.98	32	30
*10/13/92	2400	140	1000	308	2480	880	39	14.4	336	36	0.017	0.013	7.95	8.66	28	30
*10/14/92	23	7.2	20	4	196	28	8	4	6	0	0.069	0.047	7.86	7.91	32	32
10/19/92	51	0.8	93	1.6	134	0	4	1.6	0	17	0.003	0.006	7.93	8.05	33	34
10/20/92	65	44	76	42	95	27	2.4	4.8	31	346	0.007	0.017	8.15	8.17	33	33
*10/21/92	4	75	1.6	36	2.4	47	1.6	1	22	16	0.006	0.017	8.23	7.98	33	34
*10/26/92	0.8	0	4.8	0.8	8	1.6	0.8	0.8	2	6	0.024	0.004	6.83	8.22	34	34
10/27/92	5.6	7.2	7.2	5.6	8	5.6	3.2	4.8	3	10	0.025	0.113	8.08	8.27	32	32
10/28/92	0	0.8	1.6	0.8	0	0	1.6	2.4	16	10	0.007	0.013	8.17	8.23	34	33
*11/2/92	31	4.8	24	7.2	332	8.8	0	0.8	30	11	0.005	0.006	8.17	8.27	33	33
11/3/92	5.6	4.8	17.6	14.4	24	84	ND	ND	17	4	0.025	0.017	8.20	8.13	34	32
11/4/92	3.2	53	5.6	2.4	10.4	12	2.4	1.6	3	14	0.007	0.01	8.15	8.24	34	34
*11/9/92	1.6	340	4	29	1.6	31	0	0.8	6	6	0.011	0.01	8.15	8.24	34	34
*11/10/92	9.6	40	13.6	26	32	38	0	1.6	3	9	0.018	0.022	8.11	8.13	34	34
*11/11/92	52	3.2	60	3.2	100	2.4	1.6	0.8	4	4	0.005	0.001	8.09	8.06	34	33
11/16/92	1.6	13.6	1.6	2.4	8.8	3.2	0	0	24	4	0.012	0.01	8.10	8.22	34	34
*11/17/92	23	10.4	28	1.6	31	8	3.2	0	16	9	0.004	0.039	7.26	7.15	34	33
*11/18/92	2.4	3.2	15.2	6.4	19.2	16	18	0	7	10	0.013	0.003	8.11	8.24	34	34
11/23/92	0.8	0.8	20	0.8	18.4	5.6	0	0.8	20	12	0.014	0.007	8.25	8.15	34	34
11/24/92	5.6	4.8	57	22.4	24	52	0.8	0	13	7	0.001	0.004	8.11	8.27	34	34
*11/30/92	54	20.8	73	21	120	24	0	0	5	2	0.008	0.033	7.96	8.83	34	34
*12/1/92	4.8	5.6	27	9.6	34	15.2	0	0	10	7	0.04	0.014	8.81	8.60	34	33
*12/2/92	0.8	1.6	25	7.2	23	11.2	0	0	5	2	NTD	0.006	8.11	8.22	34	34
*12/7/92	4.8	5.6	16	14.4	30	16	0	0	5	146	0.003	0.004	8.18	8.25	34	34
12/8/92	11.2	2.4	8	59	69	7.2	0.8	0	2	5	0.009	0.006	8.11	8.26	33	34
*12/9/92	8.8	2.4	4	16	46	78	0	1.6	4	17	0.007	NTD	8.25	8.22	34	34
12/14/92	4.8	2.4	15.2	1.6	12.8	9.6	0	0	4	5	0.001	0.004	8.02	8.16	34	34
12/15/92	24	4.8	11.2	0.8	16.8	7.2	1	0.8	9	14	0.007	0.007	8.02	8.16	32	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 2. Kuhio Beach (Site 2) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*12/16/92	1.6	6.4	1.6	0	0.8	2.4	0.8	0	4	4	NTD	NTD	8.18	8.22	35	34
*12/21/92	4	3.2	15.2	6.4	14.4	7.2	0	0	5	12	0.008	0.001	8.07	8.16	33	32
*12/22/92	12	0.8	16.8	16.8	9.6	29	1.6	1.6	8	32	NTD	NTD	6.97	8.06	34	34
1/11/93	3.2	0.8	1.6	3.2	1.6	12.8	0	0	1	12	0.004	0.001	8.23	8.25	32	33
1/12/93	1.6	4	0	12	188	1.6	0	0	5	4	0.001	0.011	8.30	8.30	34	34
1/13/93	3.2	1.6	20	3.2	12	4	0	0	4	0	0.007	0.004	8.28	8.31	34	34
1/18/93	3	2.4	20	6.4	17.6	7.2	0	0	0	5	0.004	0.006	8.26	8.30	34	34
1/19/93	3.2	1.6	35	0	45	2.4	0	0	3	2	0.003	0.015	8.35	8.45	33	34
1/20/93	2.4	4	388	14.4	500	26.4	0	0	2	1	0.01	0.005	8.30	8.36	34	34
1/25/93	1.6	6.4	6.4	0	8.8	3.2	0	1.6	2	1	NTD	NTD	8.17	8.27	33	33
1/26/93	2.4	2.4	1.6	0	8.8	0.8	1.6	4	1	0	0.001	NTD	8.24	8.32	33	34
1/27/93	4.8	0	3.2	5.6	2.4	0	0	1.6	8	12	0.006	0.011	8.21	8.24	33	34
2/1/93	3.2	1.6	2.4	0	4.8	0	0.8	1.6	7	3	0.565	0.007	ND	ND	34	34
2/2/93	6.4	0	5.6	1.6	13.6	3.2	0	0	0	0	0.043	0.003	8.27	8.33	33	34
2/3/93	12	0	13.6	4.8	11.2	8.8	0	0	0	0	0.012	0.016	8.34	8.39	33	34
*2/8/93	0	3.2	1.6	1.6	1.6	4.8	0	0	2	1	0.004	0.009	8.34	8.34	34	34
*2/9/93	4	4.8	40	0	37	0.8	1.6	0.8	3	6	0.001	0.004	8.30	8.27	34	33
2/10/93	2.4	0	4.8	0	5.6	1.6	0	1.6	2	0	0.034	0.006	8.08	8.10	34	34
2/16/93	5.6	11.2	28	2.4	27	4	0	0	6	0	0.006	0.003	8.24	8.32	34	34
2/17/93	0	0	7.2	2.4	4.8	7.2	0	0	0	0	0.005	0.004	8.21	8.24	34	34
*2/22/93	22	4.8	0	2.4	0.8	6.4	0	1.6	2	1	0.004	0.004	8.17	8.19	34	34
*2/23/93	0	43	8	46	12	34	0	0	1	1	0.004	0.022	8.38	8.39	34	34
2/24/93	4	4.8	0.8	0.8	5.6	1.6	0	0	1	1	0.006	0.001	8.30	8.39	34	34
3/1/93	**2048	24	**1840	3.2	**2672	29	992	1.6	3	3	0.005	0.009	8.11	8.16	34	34
*3/2/93	7.2	1.6	8	4	16	4.8	0	0	2	2	0.002	0.001	8.23	8.37	34	34
3/3/93	13.6	12	9.6	4	4	0	0	0.8	15	4	0.01	0.003	8.26	8.31	34	34
3/8/93	1.6	0.8	0.8	0	1.6	2.4	0.8	0.8	3	1	0.016	0.012	8.24	8.25	33	34
*3/9/93	4.8	0	2.4	3.2	4.8	0.8	0	0	3	0	0.014	0.011	8.24	8.32	34	34
*3/10/93	0.8	0	0	0	0.8	0	0.8	0	4	9	0.008	0.004	8.24	8.27	33	33
*3/15/93	0	1.6	0	0.8	4	2.4	0	0	2	2	0.007	0.016	8.24	8.28	34	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 2. Kuhio Beach (Site 2) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*3/16/93	5.6	0.8	6.4	0.8	20	0	0.8	0	15	29	0.01	0.013	8.24	8.34	34	33
3/17/93	0	0	0	0.8	0	0	0	0	0	1	0.03	0.007	8.27	8.31	34	34
3/22/93	0	0	0	0	0.8	0	0	0	0	2	0.006	0.012	8.2	8.26	34	34
3/23/93	0	0	132	4	132	3.2	0	0.8	4	0	0.026	0.01	8.14	8.34	34	34
3/24/93	0	7.2	12.8	0	0	20.8	0.8	1.6	0	2	0.011	0.006	8.26	8.29	34	34
3/29/93	2.4	0	22	3.2	14.4	3.2	0	0	75	3	0.013	0.006	8.24	8.32	34	34
3/30/93	0.8	1.6	0	0.8	0.8	0	0	0	1	3	0.005	0.027	8.31	8.12	34	34
3/31/93	1.6	0	1.6	0	4	0	4.8	0.8	0	0	0.023	0.012	8.23	8.3	34	34
*4/5/93	0.8	0.8	0	20	0	8.8	0	0	1	1	0.018	0.01	8.12	8.32	34	34
*4/6/93	1.6	0	80	0.8	92	4.8	0	0	1	1	0.004	0.01	7.07	8.25	34	34
4/7/93	0.8	0	0	0.8	10.4	0	0	0	1	2	0.001	0.011	8.23	8.25	34	34
*4/12/93	0	0	0	0	0	2.4	0	0	1	3	0.024	0.013	8.26	8.3	34	34
*4/13/93	0	0	7.2	0	11.2	0	0	0	69	0	0.009	0.006	8.26	8.41	34	34
4/14/93	0	1.6	1.6	0.8	0	0.8	0	0.8	1	0	0.01	0.016	8.29	8.31	34	34
*4/19/93	0.8	0	4	2.4	2.4	1.6	0	0	1	3	0.01	0.013	8.25	8.32	34	34
*4/20/93	0	0	61	0	74	0	0	0.8	0	1	NTD	0.011	8.25	8.43	34	34
*4/21/93	0	4	2.4	0.8	5.6	0.8	0	0	1	6	0.005	0.006	8.11	8.25	34	34
4/26/93	196	5.6	0	0	0.8	0	0	0	1	3	0.014	0.01	8.19	8.29	34	34
*4/27/93	12.8	0.8	0.8	0	1.6	0	0	0.8	8	2	0.081	0.023	8.2	8.16	34	34
4/28/93	8	0	0	0	0	0.8	0	0.8	5	11	0.014	0.022	8.08	8.21	34	34
5/3/93	1.6	0	4.8	1.6	7.2	8.8	0.8	0	1	0	0.005	0.007	8.21	8.28	34	34
5/4/93	0	2.4	4	0	4	2.4	0	0	2	1	0.056	0.016	7.83	8.14	32	34
5/5/93	4	0	0.8	0	1.6	0	0	0	4	6	0.095	0.011	8.11	8.29	34	34
5/10/93	3.2	0.8	0	0	0.8	0	0	0	0	2	0.012	0.007	8.23	8.34	34	34
5/11/93	0	2.4	2.4	0	0	0	0	0	2	1	0.01	0.016	8.25	8.33	34	34
*5/12/93	3.2	1.6	12.8	0	0.8	0	0	0	2	3	0.042	0.039	8.1	8.15	34	34
*5/17/93	2.4	1.6	232	73	272	524	0	0	3	7	0.01	0.016	8.08	7.94	34	34
*5/18/93	4.8	4	0.8	1.6	4.8	0.8	0	0	7	3	ND	0.002	ND	8.2	ND	34
6/1/93	0.8	0.8	0.8	1.6	1.6	1.6	0	0	7	2	0.018	0.015	8.21	8.21	33	32
6/2/93	0	0	0	1.6	1.6	1.6	0	0	0	4	NTD	0.029	8.12	7.79	34	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 2. Kuhio Beach (Site 2) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*6/7/93	0.8	4.8	0	3.2	0.8	2.4	0	0	9	7	NTD	0.007	8.14	8.19	33	34
*6/8/93	0	4	2.4	5.6	9.6	3.2	0	0	10	11	0.001	NTD	8.15	8.18	33	34
*6/9/93	0	0.8	1.6	0	2.4	0.8	0	0	0	6	0.005	0.011	8.11	8.16	34	34
6/14/93	0	0	0	1.6	6.4	2.4	0	0	9	4	0.004	0.005	8.19	8.17	34	33
6/15/93	3.2	8	11.2	0	13.6	0.8	0	0	9	10	NTD	0.004	8.12	8.13	33	34
6/16/93	0	0	0	0.8	0	8	0	0	0	1	0.008	0.032	8.14	8.16	33	34
*6/21/93	1.6	56	0	0	0	0.8	0	0	6	21	0.005	0.004	8.14	8.19	34	34
6/22/93	0	0.8	4.8	0	4	0.8	0	0	5	9	0.008	0.049	8.17	8.21	34	33
6/23/93	0.8	1.6	22	0	5.6	0.8	0.8	0.8	1	1	NTD	0.01	8.14	8.17	34	34
6/28/93	0	7.2	1.6	1.6	0.8	4	0	0	8	6	0.012	0.001	8.12	8.12	33	34
6/29/93	5.6	8	14.4	26	7.2	20	0	0	2	148	0.078	0.002	8.08	8.15	33	34
*6/30/93	1.6	7.2	28	16	38	31	0	0	0	87	0.006	0.004	8.06	8.1	34	34
7/5/93	4	4.8	0	1.6	0	2.4	0	2.4	5	44	0.005	0.01	8.23	8.3	32	33
7/6/93	0	1.6	0	0	2.4	1.6	10.4	7.2	2	165	0.005	0.007	8.17	8.23	34	34
7/7/93	0.8	1.6	0	0	0	0	10.4	9.6	6	6	0.024	0.014	8.24	8.25	34	34
7/12/93	0.8	0	0	0.8	0	0.8	0.8	1.6	8	9	0.011	0.007	8.2	8.2	34	34
7/13/93	2.4	8	2.4	0.8	4	0.8	0	0	4	1	0.008	0.011	8.14	8.06	34	34
7/14/93	4.8	3.2	7.2	5.6	8	4	0.8	0.8	1	41	0.041	0.001	8.12	8.17	34	34
*7/19/93	9.6	0	144	8	84	14.4	0	0	7	0	0.007	0.006	8.12	8.24	33	34
*7/20/93	2.4	4	0	0	0	0	0.8	0	7	4	0.005	0.002	8.15	8.22	33	34
7/21/93	0	0	2.4	0	5.6	1.6	0	0	1	2	0.011	0.008	8.14	8.17	34	34
*7/26/93	42	10.4	21	27	21	71	0	0	11	2	NTD	NTD	8.28	8.31	34	34
*7/27/93	15.2	23	16.8	30	12.8	24	0.8	0	30	11	NTD	NTD	8.25	8.28	35	34
8/2/93	20	12	10.4	2.4	12.8	2.4	0.8	0	10	14	NTD	NTD	8.18	8.23	34	34
8/3/93	8.8	3.2	120	0.8	340	5.6	6.4	0	5	5	0.006	0.005	8.24	8.24	34	34
*8/4/93	0.8	2.4	0	0	0	1.6	0	0	5	285	NTD	0.004	8.12	8.17	34	34
8/9/93	12.8	8.8	2.4	3.2	8.8	0	0	0	50	25	0.17	NTD	8.15	8.18	34	34
*8/10/93	3.2	5.6	11.2	24	8.8	16.8	0	0	47	43	0.01	NTD	8.11	8.14	34	34
8/11/93	17.6	20	36	0.8	32	9.6	0	0	19	148	NTD	NTD	8.1	8.15	33	34
*8/16/93	0.8	0	0.8	3.2	6.4	3.2	0	0	13	104	0.005	NTD	8.03	8.04	33	33

*Rain obeserved within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 2. Kuhio Beach (Site 2) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*8/17/93	10.4	8	3.2	24	6.4	26	0	2.4	9	19	NTD	0.003	8.14	8.18	34	34
8/18/93	5.6	8	2.4	6.4	10.4	7.2	0	0	1	7	0.01	0.003	8.12	8.18	35	37
*8/23/93	3.2	3.2	6.4	0.8	9.6	5.6	1.6	0	5	2	0.001	ND	8.1	ND	34	ND
8/24/93	0	5.6	0.8	18.4	4	28	0	0	9	81	0.001	0.008	8.16	8.12	34	34
8/25/93	ND	1.6	ND	2.4	ND	8	ND	0	3		ND	0.027	ND	8.17	ND	34
*9/13/93	2.4	3.2	0	2.4	0.8	30	0	0.8	6	16	0.003	0.003	8.18	8.27	34	34
*9/14/93	0.8	0.8	0	0.8	8	8.8	0	0	2	5	NTD	NTD	8.31	8.2	34	34
*9/15/93	0.8	1.6	0.8	0	0.8	4	0	0	5	2	0.003	0.011	8.17	8.22	34	34
*9/20/93	1.6	2.4	0.8	24	3.2	39	0	0	19	16	NTD	0.002	8.19	8.18	34	34
*9/21/93	36	0.8	4	0	3.2	4	0	0	11	4	NTD	0.001	8.17	8.18	34	34
9/22/93	8	5.6	1.6	2.4	1.6	1.6	0	0	10	3	0.003	NTD	8.07	8.27	34	35
9/27/93	1.6	20.8	2.4	0	1.6	4	0	0	12	22	0.017	0.008	8.19	8.21	34	34
9/28/93	3.2	0.8	2.4	0	4	1.6	0	0	9	33	0.007	0.004	8.07	8.12	34	34
9/29/93	ND	ND	5.6	5.6	8.8	4	0	0	2	20	0.013	0.007	8.09	8.16	34	34

*Rain observed within 24hrs
**Estimated from maximum CFU countable
ND-Not Done
NTD-Not Detected

Appendix 3. Kuhio Beach (Site 5) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
6/8/92	0	ND	2	ND	1.6	ND	0	ND	2	ND	NTD	ND	ND	ND	34	ND
6/17/92	0	ND	46	ND	112	ND	0	ND	2	ND	0.051	ND	ND	ND	ND	ND
6/29/92	0	ND	0.8	ND	0.8	ND	0	ND	94	ND	0.025	ND	ND	ND	34	ND
*7/8/92	0	ND	0	ND	19.2	ND	0	ND	14	ND	0.017	ND	7.10	ND	32	ND
7/15/92	1.6	ND	0.8	ND	0	ND	0	ND	8	ND	0.008	ND	8.01	ND	32	ND
7/22/92	0.8	ND	0	ND	2.4	ND	0.8	ND	0	ND	0.015	ND	7.88	ND	34	ND
*7/29/92	4	ND	74	ND	96	ND	0	ND	3	ND	0.002	ND	8.25	ND	32	ND
8/11/92	0.8	ND	0	ND	0	ND	0	ND	3	ND	0.006	ND	ND	ND	34	ND
8/18/92	2.4	ND	0	ND	8	ND	5.6	ND	5	ND	NTD	ND	8.31	ND	ND	ND
8/26/92	2.4	ND	24	ND	30	ND	0	ND	2	ND	0.006	ND	8.21	ND	34	ND
8/31/92	0	ND	0	ND	3.2	ND	0.8	ND	64	ND	0.004	ND	8.12	ND	32	ND
9/1/92	0.8	ND	0	ND	2.4	ND	0.8	ND	12	ND	0.017	ND	8.05	ND	33	ND
*9/3/92	68	ND	68	ND	62	ND	1.6	ND	0	ND	NTD	ND	8.14	ND	33	ND
*9/4/92	38	ND	1.6	ND	8	ND	0	ND	9	ND	0.004	ND	8.01	ND	32	ND
9/5/92	1.6	ND	0.8	ND	6.4	ND	0.8	ND	198	ND	0.061	ND	8.07	ND	32	ND
9/7/92	1.6	ND	2.4	ND	188	ND	0.8	ND	1	ND	0.005	ND	8.19	ND	34	ND
9/8/92	2.4	ND	3.2	ND	9.6	ND	0	ND	ND	ND	0.009	ND	8.18	ND	36	ND
9/9/92	2.4	52	1.6	1.6	1.6	4	1.6	0.8	1	3	0.027	0.006	8.02	8.20	35	35
9/10/92	0	39	0.8	34	2.4	42	0.8	0	2	13	ND	ND	ND	ND	ND	ND
*9/12/92	53	ND	552	ND	776	ND	1.6	ND	37	ND	0.049	ND	8.10	ND	ND	ND
*9/14/92	73	ND	72	ND	68	ND	0.8	ND	11	ND	0.002	ND	7.99	ND	35	ND
9/16/92	212	ND	288	ND	500	ND	0.8	ND	14	ND	0.004	ND	8.05	ND	35	ND
9/18/92	8	ND	18.4	ND	22	ND	0	ND	7	ND	0.023	ND	8.07	ND	ND	ND
9/21/92	1.6	10.4	1.6	11.2	1.6	18.4	0	0	2	12	0.01	0.007	7.95	8.11	ND	33
9/23/92	0.8	5.6	13.6	3.2	2.4	8.8	0	0	6	7	0.006	0.009	8.15	8.20	32	32
9/25/92	11.2	8.8	2.4	0.8	6.4	0	0	1.6	0	3	0.061	0.06	8.08	8.20	32	32
9/28/92	9.6	1.6	4.8	0.8	4.8	2.4	0	0	19	8	0.003	0.01	8.02	8.19	34	34
9/29/92	33	1.6	38	1.6	33	3.2	0.8	0	18	5	NTD	0.001	8.08	8.23	34	34
9/30/92	5.6	4	24	4.8	27	5.6	0	0	5	4	NTD	0.002	7.94	8.23	32	34
10/5/92	0.8	3.2	3.2	0	0	0.8	0	0	12	5	0.008	0.002	7.85	8.21	32	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 3. Kuhio Beach (Site 5) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
10/6/92	ND	0	ND	12	ND	0.8	ND	0		4	ND	0.003	ND	8.13	ND	34
*10/7/92	1.6	0	28	0	24	1.6	0	0	4	3	0.031	0.03	7.46	8.38	34	34
10/12/92	ND	ND	800	1520	1780	2900	15.2	8	25	28	0.013	0.015	7.88	8.05	30	32
10/13/92	6120	168	160	520	4240	240	176	28	92	49	0.042	0.027	7.89	8.57	28	30
10/14/92	412	420	1200	320	3840	2560	13.6	13.6	5	2	0.021	0.014	7.87	8.26	30	31
10/19/92	13.6	38	7.2	35	8	49	1.6	2.4	5	1	0.005	0.001	8.02	8.03	32	33
10/20/92	5.6	38	11.2	42	135	57	2.4	6.4	39	234	0.007	0.012	8.19	8.22	34	33
10/21/92	3.2	4.8	0	0.8	1.6	3.2	1.6	0.8	14	11	0.017	0.004	8.25	7.58	34	33
10/26/92	107	7.2	38	12.8	71	9.6	1.6	0	9	6	0.01	0.013	8.17	8.28	34	26
10/27/92	5.6	20	14.4	68	30	96	1.6	1.6	4	45	NTD	0.013	8.16	8.33	32	30
10/28/92	53	13.6	416	108	312	144	5.6	2.4	27	14	0.023	0.003	8.19	8.25	33	34
*11/2/92	208	19.2	156	1040	420	520	4.8	3.2	12	20	0.012	0.011	8.19	8.24	34	32
11/3/92	20	208	4.8	60	360	68	ND	ND	5	28	0.01	0.017	8.14	8.11	33	33
11/4/92	10.4	16	40	16	31	19.2	0	0	12	10	0.007	0.007	8.17	8.22	34	34
*11/9/92	59	29	88	47	128	236	3.2	2.4	5	7	0.015	0.005	8.21	8.23	33	33
11/10/92	29	5.6	40	7.2	55	4	0	0.8	8	2	0.008	0.021	8.15	8.29	34	33
11/11/92	480	240	1476	768	1936	616	3.2	5.6	10	21	0.029	0.005	8.09	7.93	32	32
11/16/92	0.8	10.4	1.6	20	2.4	17.6	0	1.6	13	2	0.006	0.004	8.19	8.18	34	34
11/17/92	4.8	49	1.6	120	4	220	0	0	17	10	0.037	0.01	7.28	7.90	34	33
11/18/92	34	0	1.6	57	5.6	69	0	2.4	0	15	0.002	0.043	8.14	8.20	34	34
11/23/92	25	8.8	64	16.8	100	27	0.8	0.8	22	20	0.017	0.006	8.25	8.17	34	34
11/24/92	18.4	11.2	47	49	11.2	116	1.6	0.8	12	9	0.007	0.006	8.12	8.30	34	33
11/30/92	11.2	39	54	7.2	65	104	0.8	0	3	4	0.001	0.009	7.75	7.54	34	34
*12/1/92	2.4	36	29	148	37	160	0	1.6	9	10	0.011	0.007	6.84	8.04	34	33
*12/2/92	0	2.4	4	2.4	6.4	29	0	0	4	3	NTD	0.001	8.21	8.18	34	34
*12/7/92	17.6	44	32	9.6	32	12.8	0	0.8	1	4	0.002	0.007	8.22	8.26	34	34
12/8/92	6.4	20	17.6	34	45	17.6	0	0	7	2	0.009	0.01	8.16	8.16	33	34
*12/9/92	27	62	59	45	46	78	0.8	4	9	6	0.007	0.011	8.23	8.30	33	33
12/14/92	35	136	27	88	43	120	0.8	3.2	9	14	0.003	0.026	7.86	8.10	34	34
12/15/92	36	49	21	332	22	540	0	5.6	1	14	NTD	0.009	8.08	8.15	34	33

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 3. Kuhio Beach (Site 5) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
12/16/92	24	46	7.2	60	13.6	66	0.8	2.4	7	7	NTD	0.006	8.19	8.03	32	32
12/21/92	54	16	17.6	27	66	18.4	2.4	4	1680	30	0.009	0.016	8.14	8.17	32	32
12/22/92	25	20	26	55	156	224	0.8	4	9	10	0.003	0.012	7.35	7.51	34	34
1/11/93	64	34	100	24	38	35	5.6	2.4	1	8	0.024	0.005	8.23	8.32	32	33
1/12/93	21	2.4	4.8	13.6	20	5.6	0	0.8	8	5	0.007	0.003	8.31	8.32	34	33
1/13/93	212	36	4.4	62	288	77	2.4	1.6	11	4	0.012	0.03	8.22	8.27	32	32
1/18/93	20	4	49	5.6	36	12.8	0	0	11	3	0.008	0.01	8.27	8.33	34	34
1/19/93	13.6	4	23	1.6	29	4	1.6	0.8	8	5	0.016	0.015	8.38	8.43	30	34
1/20/93	36	160	45	57	70	90	11.2	232	2	5	0.003	0.013	8.33	8.37	34	34
1/25/93	48	28	44	61	71	252	0.8	4.8	2	2	0.005	NTD	8.25	8.25	33	32
1/26/93	74	112	300	12	400	36	2.4	0	5	4	0.014	0.004	8.22	8.36	33	33
1/27/93	65	33	27.2	160	184	104	2.4	4	281	4	0.002	0.004	8.18	8.24	32	33
2/1/93	11.2	2.4	11.2	24	11.2	13.6	0.8	0	6	3	0.007	0.015	ND	ND	32	32
2/2/93	5.6	8.8	25	1102	35	20	2.4	1.6	0	0	0.008	0.007	8.29	8.37	34	32
2/3/93	56	12	45	13.6	60	13.6	0	0	7	3	0.014	NTD	8.34	8.43	33	34
*2/8/93	41	38	100	348	160	292	3.2	6.4	11	14	0.012	0.011	8.27	8.32	32	33
*2/9/93	2600	232	7120	296	8760	528	200	6.4	12	11	0.002	0.057	8.37	8.26	34	28
2/10/93	60	46	134	56	204	66	4.8	0.8	13	100	0.01	0.012	8.07	8.11	34	33
2/16/93	3.2	5.6	48	36	48	71	0	0	32	4	0.002	0.004	8.17	8.29	34	34
2/17/93	17.6	27	11.2	21.6	32	17.6	1.6	0	1	0	0.003	0.002	8.20	8.26	34	34
*2/22/93	132	56	89	14.4	124	14.4	2.4	0	11	2	0.008	0.004	8.20	8.23	34	34
*2/23/93	79	4.8	88	2.4	228	1.6	1.6	0	3	1	0.019	0.012	8.36	8.46	33	34
2/24/93	32	58	26	51	25	46	0.8	1.6	0	0	0.017	0.011	8.32	8.37	34	32
3/1/93	7.2	3.2	12	4	20	11.2	3.2	4	3	3	0.011	NTD	8.10	8.17	34	34
*3/2/93	10.4	12.8	108	22	248	30	0.8	0	108	2	NTD	NTD	8.21	8.39	34	34
3/3/93	6	2.4	4	3.2	19.5	5.6	0	0.8	1	6	0.003	0.005	8.25	8.28	34	34
3/8/93	1.6	1.6	9.6	1.6	36	4	0.8	0	1	37	0.01	0.015	8.23	8.28	33	32
*3/9/93	1.6	1.6	132	4	164	3.2	2.4	0	3	2	0.005	0.004	8.25	8.39	33	33
*3/10/93	45	0	80	2.4	364	1.6	1.6	0.8	14	1	0.02	0.011	8.20	8.32	32	33
*3/15/93	0	0.8	4	2.4	8	3.2	0.8	0.8	3	1	0.018	0.021	8.27	8.32	34	34

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 3. Kuhio Beach (Site 5) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*3/16/93	13.6	23.2	16.8	0.8	34	14.4	0	0.8	8	4	0.005	0.014	8.24	8.39	34	34
3/17/93	66	61	276	84	124	76	2.4	4	14	18	0.039	0.042	8.24	8.17	32	33
3/22/93	7.2	0	13.6	2.4	12	1.6	2.4	0	5	3	0.026	0.017	8.27	8.31	32	34
3/23/93	0.8	2.4	4.8	4.8	16.8	3.2	0.8	1.6	3	1	0.014	0.008	8.28	8.35	34	34
3/24/93	52	12.8	37	260	128	28	1.6	0	7	7	0.018	0.021	8.26	8.35	34	34
3/29/93	27	0	3.2	0	9.6	1.6	0	0.8	5	0	0.008	0.006	8.26	8.36	34	34
3/30/93	2.4	0	3.2	0	3.2	8	0.8	0	2	0	0.007	0.01	8.26	8.20	34	34
3/31/93	0	6.4	4	9.6	4.8	9.6	0	0	0	0	0.008	0.012	8.27	8.29	34	34
*4/5/93	23	14.4	54	13.6	75	29	0	0	3	2	0.047	0.026	8.24	8.31	34	34
*4/6/93	5.6	0	29	9.6	83	8	0	0	3	2	0.011	0.429	7.84	8.26	34	34
4/7/93	14.4	9.6	1.6	6.4	5.6	0.8	0	0	2	26	0.012	0.01	8.27	8.29	34	34
*4/12/93	1.6	0	0.8	1.6	0.8	0.8	0	0	7	0	0.022	0.05	8.24	8.28	34	34
*4/13/93	6.4	0.8	2.4	2.4	22	0	0	0	3	3	0.012	0.014	8.17	8.39	34	34
4/14/93	1.6	0.8	0	1.6	0	0	0	0	2	7	0.013	0.333	8.30	8.32	34	34
*4/19/93	240	43	236	96	896	164	0	0	6	6	0.014	0.013	8.25	8.32	34	34
*4/20/93	51	0	1520	9.6	2080	5.6	0	0	1	208	0.002	0.014	8.31	8.39	34	34
*4/21/93	0.8	0	11.2	1.6	20	0.8	0	0	6	1	0.042	0.013	8.12	8.28	34	34
4/26/93	42	57	19.2	36	1.6	22	0	0.8	5	10	0.026	0.017	8.16	8.25	34	33
*4/27/93	4	12	0.8	0.8	2.4	1.6	0	0	4	2	0.036	0.017	7.98	8.13	34	34
4/28/93	2.4	5.6	2.4	0	0.8	4	0	0	7	12	0.017	0.009	8.16	8.28	34	34
5/3/93	0	0	3.2	0	2.4	0	0	0	2	0	0.013	0.014	8.25	8.31	34	34
5/4/93	0.8	8	0	0	1.6	0.8	0.8	0	3	2	0.05	0.007	7.95	6.87	34	34
5/5/93	2.4	0	0	1.6	0.8	2.4	0	0	7	6	0.03	0.026	8.21	8.31	34	35
5/10/93	3.2	7.2	2.4	0.8	1.6	2.4	0	0.8	2	9	0.014	0.011	8.10	8.31	34	34
5/11/93	15.2	2.4	0.8	2.4	0.8	0	0	0	4	2	0.013	0.012	8.23	8.32	34	32
*5/12/93	280	60	**1840	1648	1512	524	9.6	2.4	12	6	0.018	0.018	8.07	8.10	33	32
*5/17/93	4.8	3.2	8	3.2	9.6	3.2	0	0.8	4	2	0.013	0.02	8.11	7.96	30	34
*5/18/93	0.8	0.8	1.6	0.8	4.8	0.8	0	0	1	8	0.008	0.015	8.09	8.14	34	34
6/1/93	1.6	0.8	0.8	0	0	0	0.8	0	0	0	0.02	0.008	8.18	8.20	33	30
6/2/93	0	0	0	0.8	1.6	2.4	0	1.6	1	1	0.024	0.005	7.81	7.10	34	34

*Rain obeserved within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 3. Kuhio Beach (Site 5) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*6/7/93	2.4	20.8	0.8	0.8	4	0.8	0	0	1	1	0.008	0.006	8.14	8.22	34	34
*6/8/93	3.2	4.8	1.6	0	2.4	1.6	0	0	4	1	NTD	NTD	8.14	8.17	33	34
*6/9/93	3.2	4.8	0	1.6	1.6	4.8	0	0	2	1	0.012	0.015	8.14	8.18	34	34
6/14/93	0	0	0	0.8	0.8	0	0	0	10	1	0.007	0.014	8.11	8.15	32	34
6/15/93	0.8	1.6	0	13.6	0.8	20.8	0	0	1	2	0.005	NTD	8.09	8.10	34	34
6/16/93	0	0	0	3.2	4	4	0	0	0	1	0.008	0.023	8.14	8.18	34	33
*6/21/93	4	2.4	11.2	11.2	8	9.6	0	0	0	1	0.005	0.004	8.17	8.22	34	34
6/22/93	40	3.2	59	0.8	84	0.8	0.8	0	2	1	0.034	0.003	8.18	8.25	34	34
6/23/93	9.6	8.8	8.8	5.6	22	12	0	0	4	0	0.011	0.033	8.16	8.15	33	34
6/28/93	10.4	7.2	0	0.8	0	2.4	0	0	4	2	0.008	0.008	8.15	8.09	34	33
6/29/93	2.4	13.6	0	3.2	0	1.6	0	0	2	3	0.049	0.036	8.05	8.11	33	33
*6/30/93	1.6	4.8	0	3.2	1.6	2.4	0	0	1	0	0.005	0.017	8.04	8.07	34	34
7/5/93	1.6	0.8	0	0.8	4	0.8	0	0.8	7	4	0.006	0.005	8.27	8.31	33	34
7/6/93	2.4	0.8	1.6	0	4.8	0.8	11.2	26.4	15	7	0.01	0.002	8.19	8.22	34	34
7/7/93	0.8	2.4	0.8	2.4	1.6	2.4	12	0	1	1	0.021	0.006	8.20	8.27	34	34
7/12/93	9.6	4	1.6	2.4	2.4	0.8	0	0	3	1	0.005	0.004	8.15	8.16	34	34
7/13/93	0	0	1.6	0	0	0	0	0	1	4	0.022	0.013	8.12	7.97	34	34
7/14/93	2.4	0	0.8	4	1.6	5.6	0	0	5	5	0.001	0.015	8.16	8.13	33	34
*7/19/93	1.6	0.8	3.2	0.8	4	2.4	0	2.4	2	3	0.004	0.001	8.16	8.28	34	34
*7/20/93	0	0.8	0.8	0	0	0	0.8	0	1	8	0.009	0.001	8.20	8.26	33	34
7/21/93	0	0.8	0	0	0	1.6	0	0	4	16	0.002	0.012	8.15	8.12	34	33
*7/26/93	6.4	8	22	16.8	41	27	0	0	5	6	NTD	0.005	8.26	8.32	34	34
*7/27/93	4.8	5.6	8.8	22	9.6	22	0	0	5	12	0.004	NTD	8.22	8.27	33	34
8/2/93	0.8	0.8	0	0	1.6	0	0	0	9	1	NTD	0.004	8.24	8.28	34	34
8/3/93	0.8	0	3.2	0	2.4	0.8	0	0	5	5	0.001	0.005	8.19	8.31	34	34
*8/4/93	0.8	0.8	0	0	0.8	0	0	0	1	3	NTD	NTD	8.12	8.20	34	34
8/9/93	0	4.8	8.8	4.8	13.6	7.2	0	0	118	9	0.004	0.004	8.14	8.20	34	34
*8/10/93	5.6	0	4.8	0.8	0.8	0	0	0	12	5	0.007	0.007	8.10	8.15	34	34
8/11/93	2.4	3.2	2.4	0	12.8	4	0.8	0	1	7	0.007	0.011	8.12	8.16	34	34
*8/16/93	4.8	2.4	12	3.2	8	4	0	0	7	108	NTD	0.002	8.05	8.08	33	33

*Rain observed within 24hrs

**Estimated from maximum CFU countable

ND-Not Done

NTD-Not Detected

Appendix 3. Kuhio Beach (Site 5) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*8/17/93	4.8	0	15.2	15.2	22	18.4	0.8	0	6	18	0.009	0.003	8.12	8.20	34	34
8/18/93	0.8	2.4	4.8	4.8	9.6	2.4	0	0	3	1	0.004	0.008	8.10	8.22	37	39
*8/23/93	5.6	22	12	4.8	13.6	8	0	1.6	3	2	0.009	NTD	8.05	8.11	34	34
8/24/93	0.8	5.6	3.2	1.6	4	6.4	0	0	2	13	0.001	0.003	8.14	8.10	34	34
8/25/93	ND	0.8	ND	0	ND	3.2	ND	0	ND	6	ND	0.005	ND	7.98	ND	32
*9/13/93	0	1.6	0	0	0	2.4	0	0	2	5	0.006	0.039	8.21	8.24	34	34
*9/14/93	0	0	2.4	0	0.8	0	0.8	0.8	2	3	NTD	0.001	8.30	8.15	34	34
*9/15/93	0	0.8	0.8	1.6	1.6	2.4	0	0	1	3	0.008	0.014	8.20	8.15	34	34
*9/20/93	46	22	54	5.6	372	10.4	1	0	8	91	0.011	0.01	8.18	8.17	34	34
*9/21/93	3.2	3.2	9.6	9.6	4	16.8	0	1	9	13	NTD	0.005	8.15	8.14	34	34
9/22/93	13.6	69	8.8	14.4	4.8	12	0	2	6	472	NTD	0.009	8.14	8.13	34	34
9/27/93	0	0.8	0	0	1.6	0	0	0	11	19	0.01	0.01	8.22	8.21	34	34
9/28/93	0.8	0	6.4	0	3.2	0	0	0	7	3	0.01	0.009	8.20	8.06	34	34
9/29/93	ND	ND	288	136	320	188	0	0	4	140	0.008	0.011	8.12	8.20	34	34

*Rain observed within 24hrs
**Estimated from maximum CFU countable
ND-Not Done
NTD-Not Detected

Appendix 4. Queen's Surf Beach (Site 7) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
2/16/93	0	0.8	0	0	0	0	0.8	0.8	16	1	NTD	0.001	8.23	8.30	34	34
2/17/93	0	0	0.8	1.6	0	0.8	0	0	0	1	0.009	0.006	8.22	8.19	34	34
*2/22/93	0	0.8	0	0	0	1.6	0.8	0	2	3	0.007	0.006	8.13	8.09	34	34
*2/23/93	0.8	0.8	7.2	0	4.8	0	0	0	50	1	0.007	0.004	8.29	8.38	34	34
2/24/93	0	0	4.8	0.8	8.8	3.2	0.8	0.8	0	2	0.007	0.013	8.25	8.32	34	34
3/1/93	1.6	0	0	0.8	1.6	1.6	8.8	3.2	2	0	0.004	0.008	8.11	8.14	34	34
*3/2/93	0.8	0	71	0	71	1.6	1.6	1.6	4	1	0.006	0.001	8.24	8.36	34	34
3/3/93	0.8	0.8	0	0	0.8	0	0	1.6	1	1	0.002	0.003	8.26	8.32	34	34
3/8/93	11.2	2.4	0	0.8	0	0	0.8	0	2	0	0.003	0.009	8.20	8.21	33	34
*3/9/93	0	0	1.6	0.8	3.2	0	0	0.8	1	1	0.004	0.008	8.22	8.26	33	34
*3/10/93	0	0	0	0	4	0.8	0	0	1	1	0.01	0.004	8.26	8.30	32	33
*3/15/93	0	0	0	0	0.8	0	1.6	0	0	1	0.014	0.008	8.29	8.30	32	34
*3/16/93	0	0	1.6	1.6	0	0.8	0	2.4	1	31	0.005	0.007	8.29	8.35	34	34
3/17/93	0	1.6	0	0	0	0	0	0	1	0	0.012	0.049	8.27	8.31	34	34
3/22/93	56	1.6	0	0	42	0	0	0	0	1	0.011	0.005	8.19	8.21	34	34
3/23/93	20	2.4	0	8.8	4.8	5.6	0	0	0	12	0.016	0.029	8.04	8.18	34	33
3/24/93	12.8	0	0	0	0	2.4	0	0	0	1	0.013	0.008	8.20	8.23	34	34
3/29/93	0	0	0	0.8	0	0	0	1.6	1	1	0.006	0.011	8.29	8.34	34	34
3/30/93	0.8	0.8	0	2.4	0	1.6	0.8	1.6	1	2	0.006	0.004	8.33	8.40	34	34
3/31/93	0.8	0	0	0	0.8	0.8	0.8	0.8	0	0	0.012	0.004	8.24	8.28	34	33
*4/5/93	0	0	0	0	0	0	0	0	1	1	0.009	0.001	8.27	8.33	34	34
*4/6/93	0.8	0	0	1.6	0	2.4	0.8	0	2	0	0.065	0.005	7.64	8.25	34	34
4/7/93	5.6	0	0	0	1.6	0	0	0	13	2	0.006	0.012	8.10	8.20	34	34
4/12/93	0.8	0	1.6	0	0	0	0	0	2	4	0.036	0.013	8.27	8.26	34	34
4/13/93	1.6	2.4	2.4	0.8	0	2.4	0	0	1	2	0.008	0.009	8.26	8.32	34	34
4/14/93	0	0.8	0.8	0	0.8	0.8	0	0	1	2	0.022	0.024	8.29	8.28	34	34
*4/19/93	9.6	0	2.4	0.8	5.6	2.4	0	0	3	2	0.057	0.009	8.16	8.22	34	34
*4/20/93	6.4	2.4	0.8	0	1.6	0	0	0	0	70	NTD	0.011	8.07	8.36	34	34
*4/21/93	0.8	0.8	0	0	0.8	2.4	0	0	11	3	0.009	0.006	8.13	8.24	34	34
4/26/93	0	0	0	0	0	0	0.8	0	1	0	0.006	0.037	8.24	8.26	34	34

*Rain obeserved within 24hrs
ND-Not Done
NTD-Not Detected

Appendix 4. Queen's Surf Beach (Site 7) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*4/27/93	0.8	0	0.8	0	8	0	0	0	91	2	0.06	0.006	8.14	8.32	34	34
4/28/93	0	0.8	0.8	0.8	0.8	0.8	0	0	2	2	0.014	0.016	8.20	8.23	34	34
5/3/93	0	0	0	0	0	0	0	0	0	2	0.02	0.008	8.19	8.23	34	34
5/4/93	3.2	0	2.4	9.6	0	4.8	0	0	2	0	0.02	0.014	7.63	8.15	34	34
5/5/93	0	0	0	0	0.8	0	0	0	3	2	0.037	0.028	8.12	8.16	34	34
5/10/93	8.8	1.6	0	0.8	0	0	0	0	0	2	0.006	0.013	8.20	8.27	34	34
5/11/93	0.8	0	0	1.6	0	2.4	0	0	1	3	0.02	0.03	8.26	8.30	34	34
*5/12/93	3.2	5.6	2.4	12	6.4	8.8	2.4	0.8	3	1	0.014	0.009	8.10	8.14	33	34
*5/17/93	0.8	100	0	0.8	0.8	23	0	0	2	1	0.023	0.011	8.11	8.03	34	34
*5/18/93	0.8	0.8	0.8	0	2.4	0	0	0	3	2	ND	0.009	ND	8.16	ND	34
6/1/93	0.8	0.8	0.8	0	0	0.8	0	0	8	4	0.006	0.011	8.15	8.20	33	33
6/2/93	1.6	0.8	0.8	0	0	0.8	0	0	4	2	0.241	0.04	7.29	7.91	34	33
*6/7/93	0	0	3.2	0.8	3.2	0	0	0	1	1	NTD	0.01	8.16	8.20	34	34
*6/8/93	1.6	0.8	0	0	0	0	0	0	0	3	0.004	NTD	8.16	8.18	34	33
*6/9/93	0.8	0.8	0	0	0.8	1.6	0	0	4	0	0.004	NTD	8.06	8.09	34	34
6/14/93	1.6	0	0	0.8	5.6	2.4	0	0	0	1	0.005	0.002	8.12	8.13	34	34
6/15/93	0.8	0.8	0	0	0	0.8	0	0	2	1	0.002	0.003	8.10	8.12	34	34
6/16/93	0	2.4	0	0	0	1.6	0	0	1	1	0.008	0.011	8.11	8.18	34	34
*6/21/93	0	0	2.4	0	0.8	0	0	0	1	0	0.006	0.004	8.12	8.12	34	34
6/22/93	1.6	0.8	0	0	0	1.6	0	0	2	0	0.034	0.001	8.18	8.24	34	34
6/23/93	4	0.8	2.4	0	2.4	0	0.8	0	0	2	0.011	0.037	8.17	8.14	34	34
6/28/93	2.4	0	0	0	0	0.8	0	0.8	2	1	0.008	0.033	8.09	8.06	33	34
6/29/93	22	0	0.8	16	0	11.2	0	0	15	15	0.035	0.027	8.03	8.05	34	33
*6/30/93	1.6	21	0	1.6	0	0.8	0	0	0	8	0.007	0.001	8.02	8.04	34	34
7/5/93	0	0	0.8	0	0	0	1.6	0.8	3	10	0.002	0.003	8.24	8.29	33	34
7/6/93	1.6	0	0	0	0	1.6	16.8	4.8	2	5	0.004	0.006	8.20	8.21	34	34
7/7/93	0	0	0	0	0	0	16	16.8	1	4	0.006	0.025	8.22	8.15	34	34
7/12/93	1.6	0.8	0.8	0.8	0.8	1.6	0	0	4	6	0.004	0.002	8.18	8.19	34	34
7/13/93	0.8	5.6	1.6	9.6	0	15.2	0	116	5	4	0.002	0.003	8.17	8.17	34	33
7/14/93	2.4	0	0	2.4	0	8	1.6	1.6	1	32	0.009	0.006	8.14	8.15	34	34

*Rain observed within 24hrs

ND-Not Done

NTD-Not Detected

Appendix 4. Queen's Surf Beach (Site 7) Bacterial Levels, Reactive Phosphorus, pH and Salinity

	CFU/100ml										Reactive Phosphorus (mg/l)		pH		Salinity (ppt)	
	Enterococcus		E. coli		Fecal Coliform		C. perfringens		Bacillus spores							
DATE	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
*7/19/93	0	0	0	0	0.8	0	0	0	4	5	0.004	0.074	8.15	8.19	34	34
*7/20/93	0	0	0	0.8	0	0	0	0	5	3	0.003	0.019	8.13	8.11	32	34
7/21/93	0.8	0.8	0	0.8	0.8	0	0	0	1	2	0.01	0.001	8.13	8.16	33	34
*7/26/93	12	8.8	5.6	38	8	53	0.8	0	7	11	NTD	NTD	8.22	8.25	34	34
*7/27/93	0	16.8	17.6	21	25	28	0	0.8	10	13	0.003	0.001	8.25	8.29	34	34
8/2/93	0.8	0	0	0	0	0.8	0	0	3	0	0.006	0.001	8.20	8.27	34	34
8/3/93	0	0	0	0	0.8	0	0	0	1	4	0.002	0.003	8.21	8.27	34	34
*8/4/93	0	0	0	0	0	0	0	0	0	0	NTD	0.014	8.14	8.20	34	34
8/9/93	0	16.8	1.6	15.2	3.2	29	0	0	18	4	NTD	0.002	8.07	8.12	34	34
*8/10/93	4.8	46	3.2	55	4	70	0.8	5.6	59	9	NTD	NTD	8.10	8.09	34	34
8/11/93	4.8	46	15.2	27	36	47	0	0	4	7	NTD	NTD	8.06	8.07	34	34
*8/16/93	0	0	0.8	0.8	0.8	0	0.8	0.8	21	24	0.002	0.002	8.03	8.07	32	33
*8/17/93	0.8	3.2	8.8	4	5.6	5.6	0.8	0	1	5	0.005	0.002	8.14	8.16	34	34
8/18/93	4	0	4	2.4	2.4	3.2	0	0.8	1	4	0.178	0.005	8.13	8.20	35	38
*8/23/93	7.2	3.2	8.8	6.4	13.6	0.8	0.8	0.8	5	9	NTD	0.01	8.04	8.07	34	34
8/24/93	5.6	11.2	41	2.4	45	8	0	0.8	3	3	0.011	NTD	8.16	8.15	34	34
8/25/93	ND	0	ND	0.8	ND	0	ND	0	6		ND	0.004	ND	8.12	ND	34
*9/13/93	0.8	1.6	0	0.8	0.8	0	0	0	16	1	0.001	0.004	8.21	8.25	34	34
*9/14/93	0	0.8	0	0	0.8	1.6	0	0	3	1	0.001	0.001	8.30	8.16	34	34
*9/15/93	17.6	0.8	0	0	2.4	0	0	0	4	12	NTD	0.001	8.20	8.17	34	34
*9/20/93	22	50	12	0.8	8	1.6	0	0	39	21	NTD	NTD	8.15	8.19	34	34
*9/21/93	3.2	0	8	4	12	1	0	0	23	10	NTD	NTD	8.21	8.21	34	34
9/22/93	72	4	30	0	37	0.8	0	2	11	3	0.005	0.025	8.18	8.25	34	34
9/27/93	2.4	0.8	0	8	17.6	1.6	0	0	3	1	0.008	0.005	8.19	8.23	34	34
9/28/93	2.4	2.4	6.4	0	7.2	0.8	1.6	0	47	14	0.004	0.006	8.10	8.20	34	34
9/29/93	ND	ND	20	0.8	64	0.8	0	0	5	67	0.009	0.006	8.11	8.15	34	34

*Rain observed within 24hrs
ND-Not Done
NTD-Not Detected

A PILOT EPIDEMIOLOGICAL STUDY OF HEALTH RISKS ASSOCIATED WITH SWIMMING AT KUHIO BEACH

David M. Morens
Kimberly K. Roll
Roger S. Fujioka

Project Completion Report KSDS-5

March 1994

PREPARED FOR
State of Hawaii
Department of Health
Contract No.: ASO Log No. 92-613
Project Period: 1 April 1992-31 December 1993
Principal Investigator: David M. Morens

WATER RESOURCES RESEARCH CENTER
University of Hawaii at Manoa
Honolulu, Hawaii 96822

I. MOTIVATION FOR STUDY

A. Need for an Epidemiological Study. There is widespread belief in Hawai'i that the State's waters are polluted with sewage. In recent lawsuits against the City and County of Honolulu, which operates O'ahu's treatment plants, Hawai'i residents have claimed illnesses resulting from swimming in O'ahu's recreational waters. These claims are difficult to evaluate without information on public health risks. But at present no studies of health risk are underway, nor to our knowledge are any being planned. Moreover, there may be potential sources of water contamination or even health risks other than sewage discharge, including soil and storm drain runoff, animals, and contamination of beach waters by high bather densities.

Other data suggest that indicator organisms are poor markers of sewage contamination and of health risk, inasmuch as they may be found in environmental sources that have not been contaminated with sewage. Furthermore, the same organisms alleged to indicate sewage contamination are found in storm drains that are not contaminated with sewage. Reasonable questions arising from this situation include what originating sources indicator organisms actually reflect, whether they indicate a health risk independent of their source, and whether, in Hawai'i, they are of any value in monitoring health risks. No matter how many studies of "water quality" are completed, public health decision-making will remain clouded by controversy until the actual human risk posed by Hawai'i's marine waters has been scientifically measured. While standard measurements of the impact of sewage discharge can have important ecologic and aesthetic implications, and while measurements of indicator organisms may at best allow crude (but conceivably erroneous) inferences about health risks, only studies of exposed humans can potentially resolve the ultimate controversies surrounding Hawai'i's waters. Epidemiological study of health risks posed by ostensibly contaminated waters is thus, in our opinion, the single most important type of study that needs to be undertaken.

Evaluation of marine health risks associated with the outfall of a storm drain, into which sewage is not released, constitutes an important opportunity to examine some of these questions by controlling for sewage contamination. The ultimate aim of this type of research, for which the present study is a pilot evaluation, is thus to identify and measure those risks, and to provide the public and public officials with accurate data that can serve as a basis for informed discussion and public health decision making.

B. Assessment of Previous Water Quality Studies. The waters of Mamala Bay, including in some cases waters near the sites of this study, have for many years been monitored by City and County of Honolulu, the State of Hawai'i, and various research investigators. Extensive data collected over prolonged periods document water quality, including study of bacterial indicator organisms. However, as noted, microbial organisms assayed as water quality indicators may have little or no relationship to health risks. One problem is that many of the indicator organisms are not human pathogens. The notion, implicitly endorsed by the Environmental Protection Agency (EPA), that counts of either non-pathogenic or potentially-pathogenic indicator organisms in seawater directly correlate with potential health risk may not be valid. The studies

that seemed to generate this notion are flawed methodologically and have unfortunately been subjected to considerable over-interpretation (vide infra).

Of significance, the environmental conditions in Hawaii differ from those of the continental USA. In Hawai'i it has been shown that the same organisms regarded as indicating sewage contamination of seawater are found in high concentration in soil and streams. In fact, freshwater streams and canals, as well as ocean water fed by such sources, may typically contain higher levels of indicator organisms than seawater allegedly contaminated by sewage outfalls. For example, a 1990 report of co-investigator R. Fujioka (Hawai'i Department of Health Contract No. 88-465 [WQ/P-1]) showed that while Hawai'i's recreational beach waters were largely free of microbial indicator organisms, fresh water streams and brackish waters such as canals and lagoons contain them in high numbers: nearly half of all freshwater sources sampled by Fujioka's team had > 2,000 coliforms and/or > 1,000 E. coli per 100 ml GMT.

Although Hawai'i's recreational standard has changed from fecal coliform to enterococci, this new indicator may be no better: the same data showed that the great majority of fresh water sources in Hawaii (79%) had greater than 35 enterococci per 100 ml GMT, more than 5 times the safety ceiling set by the State (7 per 100 ml). Most had extremely high levels, as many as 6,000 to 7,000 per 100 ml GMT. Preliminary data suggest that storm drain run-off is also heavily laden with indicator organisms. Thus Hawai'i lacks not only data on human health risks from marine recreational waters, but a reliable way to distinguish between contamination by sewage and other sources of microbial indicator organisms. This distinction is critical because without knowledge of the source of indicator organisms there can be no rationale for developing public health interventions.

Subsumed under the larger question of the safety of Hawai'i's waters are other questions of importance. Is sewage-contamination a principal human risk, or is it even a risk at all? Do other sources of microbial contamination (e.g., soil, storm drains) pose a health risk to beachgoers? To what extent do indicator organisms reflect contamination from these various sources, and are any of them correlated with human risk? All of these questions remain unanswered in Hawai'i. Risk data from other locales are of little help in resolving these issues in Hawai'i. Over the years many studies in the United States and in other nations have looked at health risks to swimmers in marine waters, but, as discussed below, the results have been conflicting and difficult to generalize.

C. Assessment of Previous Epidemiological Studies. As early as 1953, published epidemiologic studies identified and predicted some of the difficult methodologic problems that complicate interpretation of studies four decades later. In that year, Stevenson reported a prospective study of fresh water swimmers (1) that led to two apparently contradictory conclusions: 1) that swimmers had higher incidence rates of illness than non-swimmers, and 2) that the increased risk was not associated with fecal borne infection, but with skin and respiratory infections. To most epidemiologists today this is not surprising -- persons who choose to participate in outdoor activities such as swimming are different in many ways from those who do not; these differences

may well account for illness risks irrespective of the presence or absence of infectious organisms in the water.

In most of the subsequent studies (2-22), including some that did and some that did not appear to show a risk, investigators typically failed to control or adjust for biases associated with inherently different risks of persons who chose exposure or non-exposure. For example, the most influential studies to date (those which formed the basis of EPA guidelines for marine indicator organisms) have been repeatedly misinterpreted. In the multi-site EPA study of Cabelli et al (6), swimmers had generally higher rates of gastro-intestinal symptoms (including "highly credible" symptoms) than non-swimmers, and in some cases the differences were statistically significant. However, the differences most often touted as measuring risk were based on the wrong comparisons -- swimmers v. non-swimmers at "contaminated" beaches, instead of swimmers at contaminated v. uncontaminated beaches and non-swimmers at contaminated v. uncontaminated beaches or, even more appropriately, swimmers v. swimmers and non-swimmers v. non-swimmers at the same beaches during times of both high and low contamination. Although often ignored, the EPA data show clearly that swimmers had higher illness rates even at uncontaminated beaches, suggesting that "swimming proclivity", rather than swimming in contaminated water could have explained illness risk. Moreover, when using the published figures to make the more correct comparisons in the same EPA data set -- simultaneously comparing swimmers with swimmers and non-swimmers with non-swimmers at the same beaches at times of high and low contamination, the risk differences appear to either become less pronounced or to disappear entirely. For example, although the EPA data demonstrate that swimmers had a statistically significant fourfold higher risk than non-swimmers of highly credible gastrointestinal symptoms after swimming in the Lake Ponchartrain levee at a mean enterococcus density of 495 per 100 ml ($p < 0.01$), swimmers also had a significantly increased risk when swimming in the same levee at 44 per 100 ml enterococcus mean density, and there was no difference in risk for either swimmers v. swimmers or non-swimmers v. non-swimmers during times of low and high density. The same data set shows similar problems with E. coli density correlations. Thus, while these data might fairly be used to suggest that swimmers have higher rates of illness than non-swimmers, they do not demonstrate that the risk is associated with water contamination.

Additional problems with existing epidemiologic data on health risks of swimming in sewage contaminated water are more difficult to exclude by attention to study design and interpretation. Two of the most serious potential problems are those related to bather-bather transmission and to community transmission of infectious organisms that also appear in sewage outflow. Most studies have not been able to control for the first problem: potentially deleterious effects of nearby bathers on seawater. Persons who excrete infectious enteric or skin organisms into seawater at a crowded beach may conceivably expose surrounding bathers to markedly higher titers of microorganisms than could ever be achieved by outfall drift. For example, rough calculations based on available data on sewage outfall (in this case, Honouliuli), drift, and organism decay suggest that an individual who excreted as little as 0.1 gram of fecal material containing 10^5 organisms per ml into 1 cubic meter of water would potentially expose an adjacent swimmer to from 1-10 billion-fold more infectious organisms than would a community epidemic of 100 persons excreting 100 grams of the same infectious fecal material into the

sewage system daily. Bather density may confound not only counts of organisms in the water but also background health risks: it is not hard to imagine that a crowded beach might lead not only to more organisms in the water, but also a higher risk of illness in general, as severe crowding has been associated with all types of infectious agents transmitted by the (so-called) "fecal-oral" and respiratory routes.

The second potential problem is that if infectious enteric organisms are being transmitted in the community, it is likely that they will show up in the community's sewage. Studies that attempt to examine risks of swimmers from sewage contaminated waters should ideally distinguish between illness acquired in the water and illness in the community, since the two may be highly correlated. In most of the published studies, persons who swim in a community's waters also live in the community and are exposed to the community's transmissible diseases, greatly complicating inferences about source of illness for study subjects who become ill.

There are many other pitfalls in epidemiological studies as well. At the outset, Hawai'i has few reliable data upon which to base decisions about either health risks or the potential efficacy of risk reduction measures for users of recreational waters. Obtaining such information would require well designed and well conducted studies that address Hawai'i's unique environmental situations. Thus a pilot study of beachgoers at a storm drain outfall is a small but necessary preliminary step in understanding health risks in Hawai'i.

II. GOALS, OBJECTIVES AND LIMITATIONS OF STUDY

A. Expectations of Study. This is a pilot study, necessarily limited in scope by funding and time constraints. It was accepted and clearly stated from the outset that it was unlikely that conclusive data on health risks would be generated. Therefore, the primary objective was to conduct a feasibility study, to specifically determine and measure critical parameters that would allow more refined estimates of the scope of work, and the amount of funds, required to obtain health risk data considered definitive at any pre-selected level of certainty. As components of the feasibility study, we also sought to obtain the following four types of data:

1. Unit cost data on the expense involved in obtaining and analyzing health risk information, in a form and manner that could be readily extrapolated to estimate the costs of future health risk studies in Hawaii marine waters

2. Background rates of indicator illnesses (principally gastrointestinal illnesses, ear infections, and skin conditions) in study subjects from and not from Hawai'i

3. Evaluation of certain novel methodologic "corrections" incorporated into the study design (see below), intended to overcome problems encountered with other studies of marine water health risks, including those of EPA

4. Development and assessment of an approach to marine water-associated health risk assessment that could be applied to future studies in Hawai'i

B. Additional Objectives.

1. To determine whether storm drain discharge poses a measurable health risk to users of recreational water

2. To determine whether any detected health risks are associated with indicators of water quality

3. To generate information useful to public officials and health planners concerned with health and sanitation

III. METHODOLOGY

A. Study Site and Experimental Design. The study site, Kuhio Beach, is a popular Waikiki beach into which the Kapahulu storm drain feeds (Figure 1). The water sampling sites include the enclosed portions of Kuhio Beach Site 1 and Site 2. Site 5 is directly adjacent to the outfall, near Kuhio Beach 1. Queen's Surf Beach (Site 7) was used as an "exposure control" beach. The study involves correlation of illnesses in swimming and non-swimming beach users with regularly-obtained water quality sampling from selected sites in the vicinity (Figure 1). A nearby "control" beach (Queen's Surf Beach) was also monitored, as were waters near the outfall (Site 5).

In keeping with the design of the EPA epidemiological study, water samples from the interview beaches were taken twice a day (morning and afternoon) to determine the microbiological quality of the water during the testing period, as described in Standard Methods for the Examination of Water and Wastewater (23). Concentrations of enterococci were monitored because the EPA study claimed a direct correlation between the concentrations of enterococci in recreational waters and incidence of swimming associated diarrheal diseases (6), and because enterococci are the principal indicators used in Hawai'i to estimate potential health risks.

Since this epidemiological study was conducted in Hawai'i, we decided that data applicable to Hawai'i should be incorporated. Thus, the waters were sampled for two other indicator bacteria shown to be useful in Hawai'i. The first alternative indicator was Clostridium perfringens, a more reliable indicator of sewage pollution of streams in Hawai'i than fecal coliforms, E. coli, or enterococci (24). C. perfringens was assayed using the methods as described by Bisson and Cabelli (25). The second alternative indicator was aerobic bacillus bacterium as a marker for soil contribution in water samples.

Twice-daily samples were tested for pH, reactive phosphorus, salinity, and concentrations of selected indicator organisms: fecal coliforms, E. coli, enterococci, bacillus spores and C. perfringens. These test methods are detailed in companion reoprt by Roll et al. Up to three interviewers encountered and administered face-to-face structured interviews to beach users, inquiring about health, beach use and other experiences in the prior three days. Each subject was then re-contacted by telephone three days later, to provide information on incident illnesses and subsequent exposures (see questionnaire, Appendix A). The study was thus prospective. It also attempted to control for exposures that occurred before and after the index exposure.

B. Case Definitions. Gastrointestinal illnesses were of primary interest, although illnesses of the eye, ear, nose, skin and respiratory tract were also surveyed to obtain a better overview of the epidemiology of illnesses related to the general beach environment (see Appendix A).

Gastrointestinal symptoms included vomiting, diarrhea, stomach ache nausea, gas, cramps, and anorexia. Highly credible gastrointestinal symptoms, as defined by EPA (6), include any one of the following:

(1) Vomiting
(2) Diarrhea with fever
(3) Diarrhea with disabling condition (remained home, remained in bed or sought medical advice), or
(4) Nausea or stomach ache accompanied by fever.

Otic symptoms include earache or ear infection. Eye symptoms include sore eye, discharge, itching, watering or redness. Skin symptoms include rash, exclusive of sunburn. Respiratory symptoms include sore throat, cough, and runny nose. These symptoms were defined in the marine and fresh recreational water quality studies conducted by the EPA (6). The same symptom definitions have been used in other water quality and swimming-related illness studies (17).

Categories of exposure status by study site included non-swimmers, swimmers who immersed the head but do not swallow water, and swimmers who swallowed water. As noted, each exposure category can be stratified on "swimming proclivity", and on three days prior and three days subsequent beach exposures.

In addition to the interview data and water quality sampling data (see below), the interviewers estimated hourly bathing density as follows: the number of persons in the water in a designated area of measured size, bounded by easily identified landmarks, was directly counted. Interview and microbial sampling emphasized the morning hours, when bather contamination of water should be minimal, and also peak hours, when bather contamination should be maximal.

To control for the potential effect of community transmission of organisms also found in seawater, we chose as subjects a mix of residents and non-residents (tourists). The latter subjects presumably are exposed to relatively fewer community risks since they do not live, work, or go to school in the community. Because many tourists speak Japanese, all of our interviewers were

bilingual, speaking fluent Japanese and English. Due to budget constraints, we were unable to monitor community transmission of enteric organisms.

To attempt to minimize the effects of adjacent bather variables we conducted bather density assessments four times each sampling day. We also focused on early morning interviews and samples, since the beaches are uncrowded for many hours overnight. Unfortunately, because of budget limitations, it was not possible to regularly assay for human skin organisms such as staphylococci. The study design was devised to include the following improvements:

1. control of bather density variables

2. improved correlation between epidemiological and microbiological sampling

3. inclusion of newer, more sensitive nonpathogen indicator organisms

4. improved reference group use in analyses

C. Development of Survey Questions. During the summer of 1992 a preliminary questionnaire was developed and refined. This questionnaire was put together from pre-existing questionnaires and data sets available to Naowarat Charoenca, formerly of the WRRC. This was expanded and developed by the investigators to incorporate questions elicited by other comparable studies of marine risks in the U.S. and elsewhere. An attempt was made to include questions that would elicit information directly comparable with data of the EPA studies, to allow for comparison. In September 1992 an initial study questionnaire was field-tested at Kuhio Beach. After administration to approximately 100 subjects it was slightly revised, field-tested a second time, re-revised, and then made available for use. The second set of questionnaire revisions was largely for ease of coding and data entry.

The questionnaire (Appendix A) was designed to elicit information about demographic characteristics, including place of residence, about past and present history of various symptoms of illness, and about exposures to other recreational waters in the past three days. It also elicited information on time, place, and phone numbers for the follow-up phone interview, conducted three calendar days after the initial face-to-face interview. The follow-up interview elicited information about incident illnesses and symptoms, as well as recreational water exposures that may have occurred after the first interview. The three day time intervals before and after the index exposure were designed to cover the incubation periods of the most common gastrointestinal and dermal conditions without being so long as to introduce recall biases.

The symptoms covered in the interviews included all gastrointestinal symptoms from the EPA studies (including the "highly credible" symptoms -- vide infra), as well as some not found in these and other studies. Some symptoms not associated with swimming in the EPA and other studies were deleted to shorten the interview process. In distinction to the EPA and some other studies, we did not exclude persons who used recreational waters before or after the initial

interview, but we did obtain information on their other exposures so that they could be stratified in analysis. Among the reasons for their inclusion is the fact that many Hawai'i beachgoers, including tourists, frequent beaches on one or more occasion: their exclusion would render the study more time-consuming and more expensive, because the majority of encountered subjects would later have to be dropped. It would also potentially introduce biases associated with sampling a highly unrepresentative group -- significant findings would therefore be less generalizable. More importantly, however, previous published studies (vide supra) suggest that persons who are regular swimmers may be at higher risk of illnesses independent of water contamination. The questionnaire elicited information on general frequency of swimming and beachgoing to control for this phenomenon. In addition, the study proportionally sampled local residents and tourists, an important mechanism for eliminating the possible confounding effects of community transmission of the same or similar illnesses (for reasons cited above, tourists should be at relatively lower risk of community- transmitted enteric infections).

D. Conducting the Survey. The survey was conducted by from one to three bilingual (English/Japanese) University of Hawai'i students working concurrently. Each student was trained in questionnaire administration and study methodology. Overall supervision was provided by Dr. Morens. Ms. Roll managed the students on a day-to-day basis, and co-ordinated water sampling with questionnaire administration. A graduate student team leader, Ms. Yurie Sakakibara, worked with the other interviewers to coordinate interview schedules, arranged back-up coverage when an interviewer was unavailable to work at a scheduled time, compiled and reported weekly statistics on interview completion, etc. A fourth graduate student was responsible for data entry, editing and (supervised) univariate analysis. Interviews were conducted relatively evenly throughout a 12 month year (September 1992 to September 1993). A special attempt was made to interview beachgoers within 24 hours after rainfall. The water sampling was performed twice daily, in the morning and afternoon, on each day of interviewing. Each of the interviewers regularly rotated beach sites on the same and subsequent days.

Beach users were sequentially encountered as they left the beach, or while they were at the beach. All children aged 5-19, along with all accompanying adults, and every other third adult or adult couple were encountered (in an attempt to over-sample children, who are at higher risk for many enteric infectious diseases, and who may also be more likely to swallow water while swimming). At the encounter, persons who agreed to participate (verbal informed consent -- see consent statement, Appendix B) were administered the questionnaire (Appendix A) in standardized face-to-face interview. The interview generally took about ten minutes. Participants were given Hawai'i postcards as compensation for their time. Persons who were ill with a "credible" gastrointestinal illness (see below), or who intended to leave O'ahu within the next three days, were excluded from the study.

IV. RESULTS & DISCUSSION

A. Cost Data. Based on the methods and scope of work in the Kuhio Beach study, including training, field-testing, and start-up time, we estimate that five full-time interviewer equivalents (10 graduate students each working 20 hours per week for 12 months) would generate approximately 21,600 completed questionnaires at a cost of $80,000. This compares favorably with the EPA studies, which elicited information on 25,242 subjects over a five year period, but presumably incurred a much greater cost in 1992 dollars. Cost data for microbial studies are much more highly dependent on which tests are chosen, but a reasonable sampling scheme of three days per week, with two samplings per day, for five indicator organisms, would incur an expense of approximately $50,000. Professional time, miscellaneous expenses, and overhead expenses are not included in these figures.

Using data generated from this study (vide infra), a cost algorithm for similar health risks studies is discussed below. It is apparent that with even a low background incidence rate of symptoms of interest, well-planned future studies could achieve ample statistical power to detect moderate differences (e.g., two-fold increases) in incidence rates between exposed and unexposed groups.

B. Descriptive Data on Study Participants. We administered questionnaires to 3721 persons and completed first and follow-up interviews on 2556 persons (68.8% completion rate) using 1.2 person-year of interview time (an estimated 2154 completed interviews per interviewer-year). The completed questionnaires/phone interviews provided data from 2,556 subjects, representing 7,668 person-days, or 21.0 person-years, of follow-up. There were 1,681 visits to Kuhio Beach site 1 (65.8%), 590 visits to Kuhio Beach site 2 (23.1%), 113 visits to Queen's Surf Beach (4.4%), and 172 visits to Site 5 (6.7%). The decision to "under-sample" the latter two sites was based on the need to target limited resources to the sites of greatest interest (vide infra). The participants included 51.4% males and 48.6% females. Participant ages ranged from 2 to 85 years, with the majority in the 20's and 30's. Of the 2,556 respondents, 1,334 (52.2%) were residents of Japan, 621 (24.3%) were residents of United States other than Hawai'i, 273 (10.7%) were residents of O'ahu, 20 (0.8%) were residents of other Hawai'ian islands, and the remaining 308 participants (12.1%) were from other countries, prominently including Canada (176 participants; 6.9%). The majority of the participants (1,462; 57.2%) identified themselves as being of Asian ethnicity; 878 (34.4%) identified themselves as Caucasian, 74 (2.9%) as Pacific Islander, 21 (0.8%) as Hispanic, and eight (0.3%) as black. Ninety of the remaining 110 persons (81.8%) identified themselves as being of mixed ethnicity.

C. Behavioral Risk Data. Of the 2,556 subjects followed for three days, only three developed otic conditions, and only three developed dermal conditions exclusive of sunburn, which had been chosen as a "control" diagnosis (Table 1). All three who "developed" otic conditions had had otic complaints in the three days prior to beach use, as had two of the three persons who developed dermal conditions, leaving respective incidence rates of 0.00 and 0.05 cases per person-year of follow-up. Because the numbers are too small for meaningful analysis, these

conditions are not considered further. Usable data on incident illnesses fell into these separate categories: gastrointestinal illnesses, ophthalmic conditions, and constitutional/respiratory conditions. Each of these is considered separately, below.

1. Gastrointestinal Complaints. Fifty-one of 2,556 subjects (2.0%) experienced one or more gastrointestinal symptom in the three days following interview. However, a larger number (65 persons) had experienced such symptoms in the three days before interview, suggesting that the frequency of gastrointestinal symptoms following beach use was not increased (Table 1). Regarding "highly credible gastrointestinal illness" (HCGI), as defined in various EPA reports (4), we identified only one incident case, corresponding to 0.05 cases per person-year. This single individual had diarrhea and vomiting with fever, but had not swum or swallowed ocean water.

Adjusting for prevalent (before interview) gastrointestinal symptomatology in the 51 persons with one or more gastro- intestinal symptom, 34 persons experienced incident vomiting and/or diarrhea in the three days after interview, representing 1.62 incident cases per person-year. The frequency of incident illness did not differ by beach site, gender, age, place of residence, or ethnic identity (data not shown).

For further analysis we stratified study participants on whether they had, or had not, visited the same or other beaches in any of the three days prior to encounter, and examined the frequency of gastrointestinal illness ("incident gastrointestinal illness") in non-swimmers, swimmers who did not swallow water, and swimmers who did swallow water.

As the data did not differ between Kuhio Beach sites 1 and 2, nor between strata for one-time and multiple beach visitors, the two sites are combined in collapsed-strata analysis and designated as "Kuhio Beach". The frequency of "incident gastrointestinal illness" in Kuhio Beach swimmers, as defined above, was 28 cases per 1,677 beach visits, or 2.03 cases per person-year. This was more than twice as high as in non-swimmers (4 cases per 595 visits, 0.82 cases per person-year), but the difference was not statistically significant ($p=0.21$, chi square). Moreover, there was no association between swallowing water and developing "gastrointestinal illness": the risk of illness was actually lower in persons who swallowed water, 1.06 v. 2.42 cases per person-year, relative risk 2.28, though not significantly so ($p=0.72$, Fisher Exact Test). There was no qualitative or trend difference in frequency of illness by number of times (from one to four) that water was swallowed.

2. Ophthalmic Illness. Forty-one persons reported one or more ophthalmic complaint (1.95 cases per person-year). There were no significant differences in frequency of ophthalmic complaints by beach visited, gender, age, ethnic background, or residence. Only 30 of the 41 cases were incident, the remainder having been prevalent in the three days before visiting the beach. This corresponds to an incidence rate of 1.43 cases per person-year. There was no relationship between either swimming or swallowing water and frequency of ophthalmic complaints.

3. Fever and Constitutional Symptoms. Only nine of 2,556 persons (0.43 cases per person-year) reported fever in the three days following interview. As noted, one of these individuals also reported vomiting and diarrhea. All of the rest had had fever in the three days prior to interview, suggesting that their subsequent fevers may not have represented incident conditions. In any case, there was no significant relationship between swimming or swallowing water and frequency of fever in the three days after visiting the beach. When examining other "constitutional" symptoms such as headache and bodyache, there were no significant differences. Swimmers who swallowed water had a marginally increased frequency of experiencing headache in the three days after visiting the beach ($p = 0.08$, chi square).

D. Microbial Risk Data. In the EPA study (26), data were reported on microbial sampling for indicator organisms at various sites. Despite the absence of a detectable risk associated with swimming or swallowing water, we sought to correlate indicator counts with human illness on the theory that an actual risk association might exist but be "buried" in the behavioral data. (This might occur in any of several ways. For example, swimming or water swallowing might constitute a risk only if some threshold level of organisms was exceeded, or only on specific occasions such as following rainfall, or only during times of high bather density. We therefore used logistic regression to associate the ordinal exposure variables (counts of specific indicator organisms, numbers of bathers) with the categorical outcome variables of presence or absence of the human illnesses of interest. Water quality data listing concentration of all indicator bacteria at each site are summarized and discussed in companion report by Roll and Fujioka (27).

Analysis of the data show a notable lack of association of any indicator organism with risk of illness (Tables 2 and 3). Neither enterococci, fecal coliforms, C. perfringens, nor any of the other organisms we studied appeared to be correlated with illness risk. This lack of association held when studying swimmers only (Table 3), or swimmers who swallowed water (not shown). Although rainfall was associated with indicator organisms, it was not associated with human risk for any of the parameters studied. These results support the subsequent EPA study (Calderon et al., 21) which showed that when the source of the indicator between the recreational water is non-point source rather than sewage, there is no correlation with bacterial counts in water and increased incidence of human enteric diseases.

It was interesting to note some apparent correlation between bather density and staphylococcus counts in limited pilot sampling at the beginning of the study. However, as noted above, we were unable to continue staphylococcus sampling throughout the study period. In any case, logistic regression analysis revealed no association between bather density and risk of gastrointestinal illness, constitutional symptoms, or eye disorders (as defined above; Tables 2 and 3, summarized in Table 4).

V. SUMMARY, CONCLUSIONS & RECOMMENDATIONS

A. Feasibility and Cost Considerations for Related Health Studies. The pilot epidemiological study provided important feasibility information on assessment of human health risks in Hawai'i waters. The information in this study was collected in what we believe to be a highly cost-effective manner, although we have no comparison figures to support this belief. Nevertheless, it is apparent that even with economical methodology, detection of relatively rare health outcomes associated with common exposures becomes prohibitively expensive as the desired level of certainty regarding study validity is increased. This is demonstrated in Table 5, which provides crude cost estimates, in 1992 dollars, for health risk studies of the type conducted here. The Table is constructed to provide cost estimates for studies conducted under pre-specified scientific expectations, which must be chosen, from among many options, beforehand.

In reading the Table, it is helpful to think of the first three columns as representing preliminary decisions about the features of the study, and the last four columns as estimates of the scope of work required as a consequence of these choices. The scope of work is thus dictated by the features that have been chosen: it is represented in the Table by the numbers of subjects required to meet the study requirements and, consequently, the study cost.
The first column records the magnitude of the risk difference between exposed and non-exposed persons (e.g., swimmers v. non-swimmers) this study will try to detect, and is selected based on public health perceptions of the importance of a high v. low "attributable risk per cent". For example, if public health officials feel that only a doubling of gastroenteritis risk for swimmers would be of sufficient concern to warrant public health action, then the 100% category might be chosen. If, on the other hand, it was felt that even a 25% increase in risk was sufficient to justify action, then the 25% category might be selected.

The second column represents the statistical "power" of the study to correctly detect a health risk when a health risk exists. This column could be interpreted as answering the question "If swimming at the beach really does double a person's chance of getting gastroenteritis, how likely is the study to detect this increased risk, with acceptable certainty, in a study of X people?" This is of course the opposite of asking how likely it is that a true risk difference will be missed merely because too few people have been studied. It can be seen that raising the level of certainty from only 80% to 99% more than doubles the study size and cost. "Power" thus represents a potential trade-off, and its selection may depend on the public health consequences of failing to take preventive action because a true health problem is erroneously believed not to exist.

The third column represents the "probability value" or "p value". This figure reflects the likelihood that a detected risk difference is real, and not just a chance statistical artifact. For example, one might ask the question "If our study finds that swimmers are twice as likely as non-swimmers to get gastroenteritis, how likely is it that that difference is not real, but a fluke arising from statistical chance?" Obviously, the lower the "p value" the better, but as was true for raising statistical power, lowering the p value increases study costs considerably. The p value is selected based on public health assessment of the consequences of falsely concluding that a risk

exists when, in reality, it does not. When public health action is required, and especially when large expenditures would be involved, the lowest p value possible is desirable.

From the first three columns of Table 5, it can be seen that a number of public health considerations need to be factored into any determination of whether it is desirable to conduct a health risk study and, if it is, what study size to recommend. Ideally, any study undertaken would be highly able (power, column 2) to detect even a small risk increment (column 1) with reasonable assurance (column 3) that the detected risk was real. Unfortunately, satisfying all three of these criteria, for water risk assessment or for any study of uncommon conditions, may be extremely expensive.

Certain other considerations should also be pointed out in cost assessment calculations. First, the figures used in Table 5, provided by this pilot study, are situation-specific. The unit cost of completing an encounter and follow-up interview, including data entry and analysis, was estimated at $3.70 in direct costs. This cost might vary considerably in other studies, depending on such factors as availability of subjects, length of the questionnaire, required level of training and experience for interviewers, etc. It is our opinion that it would be difficult to achieve or reduce this figure in other studies because, in an effort to overcome the expected problem of insufficient funds to achieve optimal sample size, we used an abbreviated questionnaire, concentrated on an extremely crowded beach where potential study subjects were nearly always available, even after rains, and hired and trained comparatively inexperienced student interviewers. Furthermore, in part for methodologic reasons (see below), we used a three day follow-up time, shortening administration of the second questionnaire, and reducing the number of subjects lost to follow-up.

An even more important consideration is that the sample size calculations used to provide estimates in column 4, Table 5, were taken from background risk figures generated by this study. To be as accurate as possible in estimating what is ultimately unknowable, sample size calculations require a best guess of the baseline risk of the disease in question. When multiple outcomes need to be detected, the sample size must normally be further increased. We selected an intermediate background incidence estimate (i.e., neither conservative nor generous) of about 15 incident cases per 2,556 persons, the approximate risk for serious but not "highly credible" gastrointestinal symptoms in non-swimmers (0.0087), and also for swimmers who swallowed water (0.0087), before correction for prevalent illnesses. Such figures may or may not be representative of risks associated with other situations. For most of the incident outcomes we sought to detect, relatively few cases were ultimately identified. For example, we found only about 30 persons with even loosely-defined gastrointestinal illnesses. Sample size estimates vary widely as the background occurrence of the disease varies. Thus the sample size required to detect risk associated with a "healthier" site would be comparatively larger than the sample size needed to detect risk associated with a "less healthy" site. A reasonable estimate of the "background risk" of a particular study site is perhaps the single most important factor in estimating study size, scope and cost. It should also be pointed out that the figures in Table 5 do not include indirect or overhead costs, include only nominal professional fees, and are expressed in 1992 dollars.

B. Risk to Swimmers at Kuhio Beach. We found no evidence of human health risk associated with recreational use of Kuhio Beach waters known to receive discharge from the Kapahulu Storm Drain. The results clearly indicate that human health risk was not high. However, it should be obvious from the above discussion, that it cannot be concluded with certainty that no health risk exists. There are several reasons for caution in interpretation of these data.

First and perhaps the most important reason is that the study may not have been large enough to detect a health risk that was real, but small. While it may be reassuring that this study appears to rule out the possibility of a major health risk, it is still possible that swimmers in Kuhio Beach waters are at low but increased risk that would only be detectable with larger studies. As can be seen from Table 5, even if our study size had been more than tripled (with an approximate tripling of cost), there would still have been a 20% chance of missing a doubling of gastroenteritis risk for swimmers (albeit from extremely low risk to very low risk), under the assumption of the generic background rate we originally selected (15 cases per 2,556 beachgoers). As it was, we observed a slightly higher background rate than the one selected, and can therefore be about 90% certain that risk of gastroenteritis for beachgoers was not increased two-fold or more, about 95% certain that the risk for swimmers was not increased by two-fold or more, and about 95% certain that the risk for water-swallowers was not increased by two-fold or more. However, since the background rate of illness can never actually be known until the study is finished, to ensure that studies will generate usable results it is normally necessary to select a conservative estimate for the purpose of study size calculations, thereby increasing the expected cost. In some situations, however, a sequential occurrence algorithm can be set up in order to terminate the study if the desired level of certainty is reached earlier than expected based on conservative planning assumptions.

Secondly, because of limited resources we decided to focus more on Kuhio Beach and less on the comparison beach (Queen's Surf). The latter beach was generally much less crowded, making it more difficult and more costly to enroll subjects at that site. Furthermore, although we would have liked to enroll more subjects swimming in the Site 5 area, few persons entered or swam in that area, limiting availability of subjects. As a consequence, the majority of subjects were from Kuhio Beach Site 1 and Site 2, reducing the possibility of detecting risks in outfall v. non-outfall water in favor of the more important, but incomplete, comparison of risks at times of high and low organism counts at the same sites. We consider this an unfortunate but necessary trade-off.

Thirdly, although we did consider "bather density" we were unable to monitor skin organisms such as staphylococci. Since humans harbor, exude, and excrete high concentrations of potentially pathogenic skin and enteric organisms into the water, it would seem plausible that any risk associated with swimming in crowded waters would be more likely to come from adjacent swimmers than from distant environmental sources. However, this possibility could neither be studied nor controlled, except in a crude way with density estimates.

Fourthly, it is possible that true microbial risk was periodic and therefore submerged in the low background risk. This might theoretically occur in any of several ways. Risk associated

with only a high threshold of indicator organisms would escape detection, as could risk not associated with indicator organism counts. It is also conceivable that some particular combination of physical parameters we did not specifically look for (e.g., rainfall, pH, and tide) was associated with a true but submerged risk. These kinds of situations would be difficult or impossible to detect in any scientific study.

Finally, we were unable to control for community transmission of organisms. However, we doubt this harmed our study for two reasons. Since the Kapahulu Storm Drain presumably does not contain sewage, there would be at best limited opportunity for such human pathogens to enter it. Also, we encountered primarily tourists, who presumably had less contact with prevalent endemic organisms than persons who live and work in the community. We found no differences whatsoever, in any parameter, between tourists and local residents.

C. Evaluation of Study. Among the unique aspects of this study, we followed persons for only three days, as compared to eight to 10 days in the EPA studies. It might be argued that our study could have missed detecting a true risk by selecting an artificial cut-off time to detect incident illness that was less than the incubation period for many of them. Aside from trade-off considerations in conducting a study with limited resources, we believe the three day cut-off period is justified and possibly optimal. It is widely recognized that while many different types of organisms may cause waterborne illness, the principal cause is a group of viruses known as "Norwalk agents" (apparently enteric caliciviruses). These viruses are associated with an incubation period of 12-36 hours. Thus virtually all incident Norwalk disease, as well as some, but not all, other diseases associated with swimming, would be picked up in our three day interval. Furthermore, the three day period greatly limits the possibility and magnitude of recall biases, which plague virtually all health studies that rely on subjects' memories. Recall biases appear to rise substantially as the duration of the recall period increases, and are particularly problematic for persons who have developed illnesses. Such persons may differentially recall exposures ("rumination bias"). Finally, the three day period allowed us to enroll many tourists; most Japanese visitors to Hawai'i, and tourists from many other places as well, stay on O'ahu for only a few days. Specifying long follow-up periods would have forced us to impose highly restrictive selection criteria on beachgoers, limiting the generalizability of findings, eliminating our ability to control for the potentially confounding effect of community transmission, and greatly increasing study costs.

It is interesting to note that the background rate of gastrointestinal illnesses we observed (between 10 and 20 per 1,000 persons per three day follow-up) is around 10%-50% that of the total (i.e., non-"highly credible") in the EPA studies, which were associated with follow-up periods of from two to 10 days. When we applied the EPA definition for "highly credible" symptoms, the background rate fell to about one twentieth that found in the EPA studies. The reason for this is unknown: it could be due to follow-up differences or to lower community illness rates. That the EPA background illness rates tended to be higher in long v. short follow-up could reflect incident illnesses with long incubation periods, but may more likely represent an unvarying background risk observed over a longer interval. This raises the question of whether high level endemic transmission in the EPA study communities could have confounded results

by leading to simultaneous community acquisition of infection and appearance of sewage-borne community organisms in the water, thereby falsely implicating water as the source of illness.

We generated sufficient outcome data to evaluate risks for three separate categories of illness in beachgoers: gastrointestinal illnesses, ophthalmic illnesses, and constitutional/respiratory illnesses. Of these, the category of greatest interest is the gastrointestinal illnesses, which have been epidemiologically associated with epidemics caused by eating contaminated food and drinking contaminated beverages. Furthermore, the association of many different behaviors with gastrointestinal illnesses has become fixed in the public's mind. As noted, the background rate of gastrointestinal illnesses we found in three day follow-up was lower than that found in the EPA studies. Moreover, based on a looser definition of outcome than that used in some of the EPA computations (vomiting and/or diarrhea of no other known cause), we did not detect increased risk in swimmers, nor in swimmers who swallowed water. Using the stricter of the EPA definitions, we identified only one person with "highly credible" gastro- intestinal symptoms. It thus appears that the background rate of illness in our subjects in 1992-1993 is lower than for EPA study subjects in the 1970s. While this is good news, it also means that detecting the presence or absence of water-associated health risks will likely be much more difficult and more expensive in Hawai'i; measuring those risks, if they exist, may be even more problematic.

The value of seeking to detect "highly credible" gastrointestinal symptoms in Hawai'i is questionable. Although scientists agree that highly specific case definitions are preferable to less specific definitions, which may misclassify persons with other diseases as having diseases of interest, in this situation they are probably of greater value in studies with long versus short follow-up, because after 10 days recall of minor complaints like stomach ache may be prone to significant error. On the other hand, tourists with novel vacation itineraries to prompt recall, and probably also with increased salience of somatic occurrences, would probably be more likely to accurately recall, and less likely to erroneously remember, symptoms occurring within the past three days.

It is curious to note that the incidence rate of gastrointestinal illness in swimmers was higher (though not significantly so) than in non-swimmers, while swimmers who swallowed water were at lower risk (again, insignificantly) than swimmers who did not. While it must be assumed that these findings represent statistical chance, in planning similar health studies it should also be considered that persons who swim might be at inherently different risk of illness than those who do not. To cite a purely speculative but illustrative example, swimmers might be more likely to be "athletic risk takers" and non-swimmers "cautious couch potatoes". Each of these lifestyle characterizations might, independent of any particular instance of swimming or water-swallowing, predict a different background risk of gastrointestinal symptoms. For example, the former group of "athletic risk takers" might tend to overeat, drink more alcoholic beverages, consume too many chili peppers, be less cautious in hand washing, diaper changing, etc., and thereby be at elevated risk of gastrointestinal illness before even approaching the water to swim. As discussed above, we believe this phenomenon probably operated in the EPA and some other studies, and may well have led to misinterpretation of the findings of those studies.

Skin and ear infections are well known risks of swimming, but for the most part are not thought to be associated with water contamination. Even persons who swim in highly chlorinated pool water are at risk of acquiring ear and skin infections due to skin maceration, which encourages growth of dermal bacteria and fungi that may be part of the individual's normal flora. Viral conjunctivitis has occasionally been associated with swimming pool outbreaks in which chlorination has lapsed, but is not commonly thought to be waterborne and, like skin and eye infection, would be suspected of resulting from autoinfection rather than exogenous marine organisms. Constitutional symptoms are by nature non-specific and therefore make poor outcome indicators for studies of this type. The constellation of fever, headache, bodyache, and respiratory symptoms is characteristic of influenza and many influenza-like illnesses, but few are thought capable of water-borne transmission. Thus, it is not surprising that the risk of these conditions was not greatly different in swimmers. It is of interest that the three-days frequencies of the individual and composite symptoms in these categories were about half those detected in EPA studies with eight to 10 day follow-up. We found no evidence to suggest that either these symptoms or related illnesses would be of value for outcome monitoring.

In conclusion, this epidemiological pilot study generated realistic cost estimates for possible future studies of this type, and also indicated that no major health risk to swimmers appeared to exist. While this study could not, because of its size and scope, completely rule out all health risks, it was suggested that such risks, if they exist, must be very small. We hope that these data, interpreted in conjunction with other public health data on the severity, impact, preventability, and priority of illnesses associated with potentially water-borne microorganisms, will be useful to health officials and policy makers.

Acknowledgements: We thank Andrew Grandinetti for assistance in statistical analysis; Etsuko Chida and Achara Thawatwiboopol also assisted in data entry and analysis. Interviews were conducted by Etsuko Chida, Atsushi Nishihata, Yurie Sakakibara, and Satoru Yamamoto.

REFERENCES

1. Stevenson AH. Studies of bathing water quality and health. Am J Publ Health 1953;43:529-38.

2. Moore B, Blowers AR, Crone PB, et al. Sewage contamination of coastal bathing waters in England and Wales. A bacterial and epidemiological study. J Hyg 1959;57:435-71.

3. Cabelli VJ, Levin, MA, Dufour AP, McCabe LJ. The development of criteria for recreational waters. In: Discharge of Sewage From Sea Outfalls, London: Pergamon Press; 1975;63-73.

4. Cabelli VJ, Dufour AP, Levin MA, McCabe LJ, Haberman PW. Relationship of microbial indicators to health effects at marine bathing beaches. Am J Public Health 1979;69:690-6.

5. D'Alessio DJ, Minor TE, Allen CI, Tsiatis AA, Nelson DB. A study of the proportions of swimmers among well controls and children with enterovirus-like illness shedding or not shedding an enterovirus. Am J Epidemiol 1981;113:533-41.

6. Cabelli VJ, Dufour AP, McCabe LJ, Levin MA. Swimming-associated gastroenteritis and water quality. Am J Epidemiol 1982;115:606-16.

7. Mujeriego R, Bravo JM, Feliu MT. Recreation in coastal waters: Public health implications. VIes Journeés Étud Pollutions 1982:585-94.

8. El Sharkawi F, Hassan MNER. The relation between the state of pollution in Alexandria swimming beaches and the occurrence of typhoid among bathers. Bull High Inst Public Health Alexandria 1982;12:337-51. [Or 1983?]

9. Foulon G, Maurin J, Quoi NN, Martin-Bouyer G. Étude de la morbidité humaine en relation avec la pollution bacteriologique des eaux de baignade en mer. Rév Franç Sci L'Eau 1983;2:127-43.

10. Seyfried PL, Tobin RS, Brown NE, Ness PF. A prospective study of swimming-related illness. I. Swimming-associated health risk. Am J Publ Health 1985;75:1068-70.

11. Fattal B, Peleg-Olevsky E, Yoshpe-Purer Y, Shuval HI. The association between morbidity among bathers and microbial quality of seawater. Water Sci Tech 1986;18:59-69.

12. Fattal B, Peleg-Olevsky E, Agursky T, Shuval HI. The association between seawater pollution as measured by bacterial indicators and morbidity among bathers at Mediterranean bathing beaches of Israel. Chemosphere 1987;16:565-70.

13. Cheung WHS, Kleevens JWL, Chang KCK, Hung RPS. Health effects of beach water pollution in Hong Kong. Proc Inst Water Env Management 1988:376-83.

14. Ferley JP, Zmirou D, Balducci F, et al. Epidemiological significance of microbial pollution criteria for river recreational waters. Int J Epidemiol 1989;18:198-205.

15. Lightfoot NE. A prospective study of swimming related illness at six freshwater beaches in Southern Ontario. Ph.D. Dissertation, University of Ontario, 1989.

16. Alexander LM. Health risks associated with exposure to seawater contaminated with sewage: The Blackpool beach survey 1990. Lancaster University Environmental Epidemiology Research Unit, 1991.

17. Cheung WHS, Chang KCK, Hung RPS, Kleevens JWL. Health effects of beach water pollution in Hong Kong. Epidemiol Infect 1990;105:139-62.

18. Zmirou D, Ferley JP, Balducci F, et al. Évaluation des indicateurs microbiens du risque sanitaire lié aux baignades en rivière. Rev Épidém Santé Publique 1990;38:101-10.

19. Balaraja R, Soni, Raleigh V, Yuen P, Wheeler D, Machin D, Cartwright R. Health risks associated with bathing in sea water. BMJ 1991;303:1445-6.

20. Brown JM, Campbell EA, Rickards AD, Wheeler D. Sewage pollution of bathing water. Lancet 1991;2:1208-9.

21. Calderon RL, Mood EW, Dufour AP. Health effects of swimmers and nonpoint sources of contaminated water. Int J Env Health Res 1991;1:21-31.

22. Brown JM, Jones F, Kay D, Stanwell-Smith R, Wyer M, Morano R. Water and non-water-related risk factors for gastroenteritis among bathers exposed to sewage-contaminated marine waters. Int J Epidemiol 1993;22:689-708.

23. American Public Health Association, American Water Works Association, Water Pollution Control Federation. Standard Methods for the Examination of Water and Wastewater, 17th Edition, APHA:1989.

24. Fujioka RS, Shizumura LK. Clostridium perfringens, a reliable indicator of stream water. J Water Pollution Cont Fed 1985;57:986-92.

25. Bisson JW, Cabelli VJ. Membrane filter enumeration for Clostridium perfringens. Appl Env Microbiol 1979;37:55-66.

26. Cabelli, V. J. 1983. Health effects criteria for marine recreational waters, EPA-600/1-80-031, U.S. Environmental Protection Agency, Washington, D.C. 98 p.

27. Roll K.K., Fujioka R.S. Microbiological characterization of the water and sediment in Kapahulu Storm Drain and Kuhio Beach. Project Completion Report KSDS-4. March 1994.

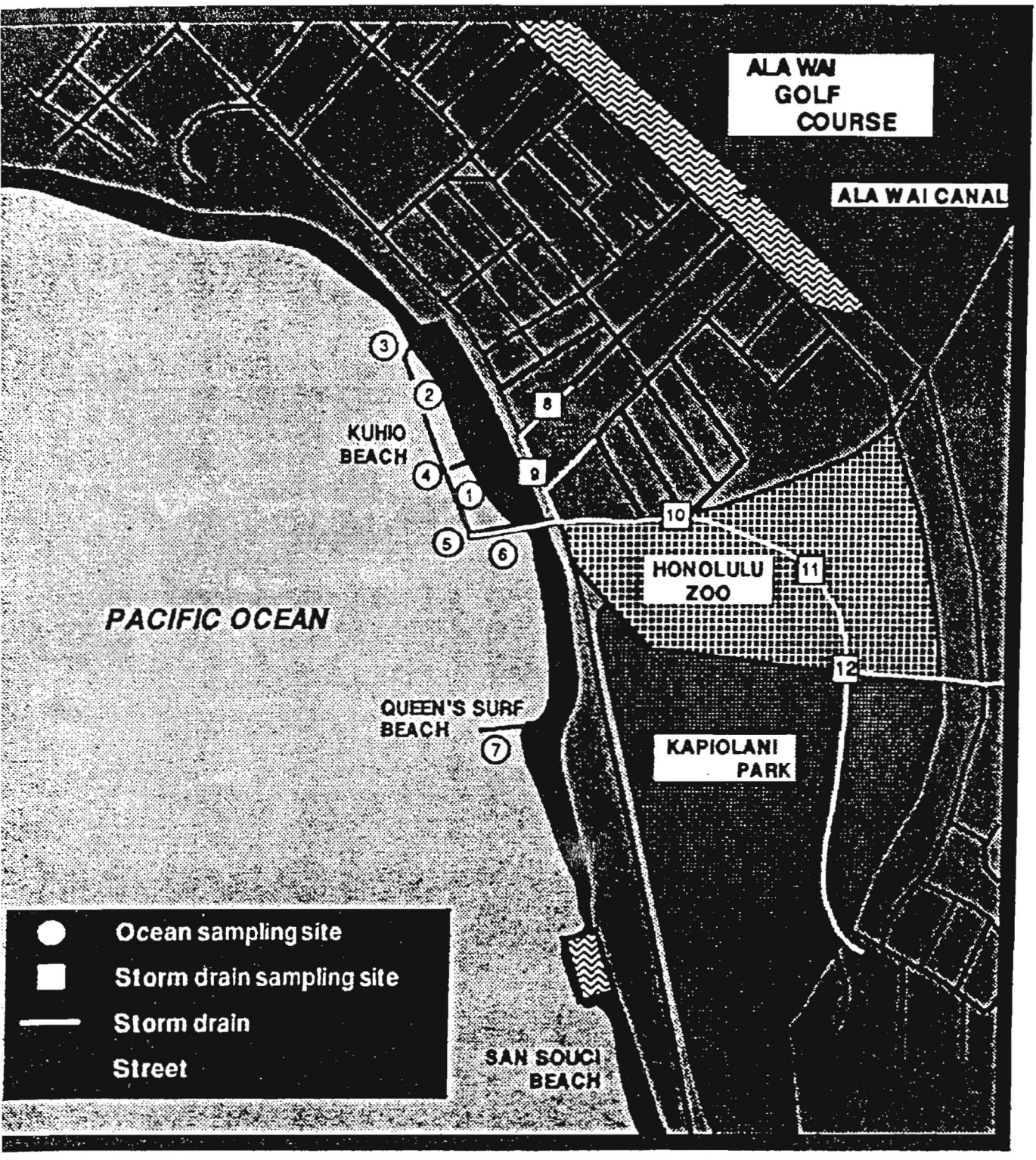


Figure 1. Sample Sites for Kapahulu Storm Drain System/Kuhio Beach Study

Table 1. Signs and symptoms in 2,556 beachgoers in the three days before and after interview, four beach sites combined, Kapahulu Storm Drain risk study, Honolulu, Hawai'i, 1992-1993.

	Three Days Before	Three Days After
Gastrointestinal		
Nausea	13	4
Vomiting	6	2
Diarrhea	27	39
Stomach ache	21	3
Cramps	5	1
Gas	26	6
Anorexia	9	4
Ophthalmic		
Red eyes	30	38
Itchy eyes	1	3
Watery eyes	6	11
Eye discharge	6	3
Eye pain	6	7
Photophobia	0	1
Optic		
Ear ache	9	2
Ear infection	3	1
Dermal		
Rash	10	3
Sunburn	63	60
Other		
Fever	15	9
Headache	88	38
Body aches	41	19
Rhinorrhea	48	28

Table 2. Association between selected indicator organisms and occurrence or gastrointestinal (GI), constitutional, and eye disorders, as defined in text, Kuhio Beaches 1 and 2, all beachgoers, logistic regression analysis, Honolulu, Hawai'i, 1992-1993

GI disorder

Indices	*beta (β)	Standard Error	Odds Ratio	Confidence Interval Lower	Upper
enterococci	0.0059	0.0091	1.006	0.988	1.024
fecal coliform	0.0044	0.0047	1.004	0.995	1.014
C. perfringens	0.0032	0.0120	1.003	0.980	1.027
E. coli	0.0055	0.0070	1.006	0.992	1.020
bather density	-0.0029	0.0065	0.997	0.984	1.010

Constitutional disorders

Indices	*beta (β)	Standard Error	Odds Ratio	Confidence Interval Lower	Upper
enterococci	0.0005	0.0016	1.000	0.997	1.004
fecal coliform	-0.0003	0.0006	1.000	0.999	1.001
C. perfringens	0.0072	0.0310	1.007	0.948	1.070
E. coli	0.0053	0.0021	1.005	1.001	1.009
bather density	-0.0035	0.0069	0.997	0.983	1.010

Eye Disorders

Indices	*beta (β)	Standard Error	Odds Ratio	Confidence Interval Lower	Upper
enterococci	0.0015	0.0044	1.002	0.993	1.010
fecal coliform	0.0004	0.0013	1.000	0.998	1.003
C. perfringens	0.0014	0.0091	1.001	0.984	1.019
E. coli	0.0008	0.0026	1.001	0.996	1.006
bather density	0.0085	0.0083	1.009	0.992	1.025

* 1 - β = power of a test

Table 3. Association between selected indicator organisms and occurrence of gastrointestinal (GI), constitutional, and eye disorders, as defined in text, Kuhio Beaches 1 and 2, swimmers who immersed heads in water, logistic regression analysis, Honolulu, Hawai'i, 1992-1993

GI disorder

Indices	*beta (β)	Standard Error	Odds Ratio	Confidence Interval Lower	Upper
enterococci	0.0168	0.0177	1.017	0.982	1.053
fecal coliform	0.0059	0.0061	1.006	0.994	1.018
C. perfringens	0.1023	0.1664	1.108	0.799	1.535
E. coli	0.0079	0.0091	1.008	0.990	1.026
bather density	-0.0029	0.0069	0.997	0.984	1.011

Constitutional disorders

Indices	*beta (β)	Standard Error	Odds Ratio	Confidence Interval Lower	Upper
enterococci	0.0007	0.0030	1.001	0.995	1.007
fecal coliform	0.0001	0.0014	1.000	0.997	1.003
C. perfringens	0.0008	0.0079	1.001	0.985	1.016
E. coli	0.0002	0.0025	1.000	0.995	1.005
bather density	-0.0038	0.0072	0.996	0.982	1.010

Eye Disorders

Indices	*beta (β)	Standard Error	Odds Ratio	Confidence Interval Lower	Upper
enterococci	0.0001	0.0014	1.000	0.997	1.003
fecal coliform	-0.0006	0.0007	0.999	0.998	1.001
C. perfringens	0.0097	0.0545	1.010	0.907	1.124
E. coli	0.0001	0.0022	1.000	0.996	1.004
bather density	0.0118	0.0090	1.012	0.994	1.030

* 1 - β = power of a test

Table 4. Logistic regression of bather density on illness outcome for three types of illnesses, Kuhio Beaches 1 and 2, Honolulu, Hawai'i, 1992-1993. The illnesses are defined above in the report.

Illness	beta (β)	Standard Error	Odds Ratio	95% Confidence Interval
Gastrointestinal	-0.0029	0.0065	0.9971	0.9845-1.0099
Constitutional	-0.0035	0.0069	0.9965	0.9831-1.0101
Ophthalmic	0.0085	0.0083	1.0085	0.9923-1.0251

TABLE 5.

Magnitude of Difference in Risk to be Detected	Probability of correctly identifying a risk when a risk does, in fact, exist 1 - β	Probability that detected risk could be due to chance alone and not to an actual risk α	Number of total subjects required N	Estimated cost of epidemiological study component (1992 dollars) At $3,702 per 1000 completed encounters	Estimated cost of microbiological study component (1992 dollars)	Total cost excluding overhead and professional services (1992 dollars)
100% (2-fold increase)	.80	.05	9,200	$ 34,058	$ 50,000	$ 84,058
	.90	.05	12,314	$ 45,586	$ 50,000	$ 95,586
	.95	.05	15,231	$ 56,385	$ 50,000	$ 106,385
	.99	.05	21,533	$ 79,715	$ 50,000	$ 129,715
	.80	.01	13,690	$ 50,680	$ 50,000	$ 100,680
	.90	.01	17,440	$ 64,563	$ 50,000	$ 114,563
	.95	.01	20,881	$ 77,601	$ 50,000	$ 127,601
	.99	.01	28,167	$ 104,272	$ 50,000	$ 154,272
50% increase	.80	.05	33,324	$ 123,365	$ 50,000	$ 173,365
	.90	.05	44,608	$ 165,139	$ 50,000	$ 215,139
	.95	.05	55,177	$ 204,265	$ 50,000	$ 254,265
	.99	.05	77,991	$ 288,723	$ 50,000	$ 338,723
	.80	.01	49,584	$ 183,560	$ 50,000	$ 223,560
	.90	.01	63,171	$ 233,859	$ 50,000	$ 283,859
	.95	.01	75,632	$ 279,990	$ 50,000	$ 329,990
	.99	.01	102,028	$ 377,708	$ 50,000	$ 427,708
25% increase	.80	.05	142,335	$ 526,924	$ 50,000	$ 576,924
	.90	.05	190,537	$ 705,368	$ 50,000	$ 755,368
	.95	.05	235,665	$ 872,432	$ 50,000	$ 922,432
	.99	.05	333,163	$ 1,233,369	$ 50,000	$ 1,283,369
	.80	.01	211,788	$ 784,039	$ 50,000	$ 834,039
	.90	.01	269,818	$ 998,866	$ 50,000	$ 1,048,866
	.95	.01	323,042	$ 1,195,901	$ 50,000	$ 1,245,901
	.99	.01	435,781	$1,615,261	$ 50,000	$ 1,663,261

1. Name _____ (Accn. No._____) | _ _ _ _ _

2. Address _____

_____

_____

_____

3. Phone #1 _____-_____ ( ) Phone #2 _____-_____( )

4. Best times to call in 3 days: _ _ _ _;_ _ _ _;_ _ _ _

KUHIO/QUEEN'S SURF BEACH
QUESTIONNAIRE

I. RECORD KEEPING DATA — Coding Column

1. Beach (1=Kuhio; 2=Queen's Surf; 3=Other_____) ____

II. SUBJECT DATA

(Accn. No._____) _ _ _ _ _

5. Gender (1=Male; 2=Female) ____

6. Age __ __

7. Main Ethnic Group (1=Caucasian; 2=Black; 3=Hispanic; 4=Asian; 5=Pacific Islander; 6=Other; 7=Unknown) ____

8. Residence (1=O'ahu; 2=Outer Island; 3=Other U.S.; 4=Canada; 5=Japan; 6=Other Asia; 7=Europe; 8=Other_____) ____

III. PAST EXPOSURES

Past 3 Days	Kuhio Beach	Queen's Surf Beach	Other Beach	Pool/Hot Tub	Stream	Pond
1. No. of times went to	__	__	__	__	__	__
2. Occasions immersed head	__	__	__	__	__	__
3. Occasions swallowed water	__	__	__	__	__	__

IV. INCIDENT EXPOSURES

Next 3 Days	Kuhio Beach (___)	Queen's Surf Beach (___)	Other Beach (___)	Pool/Hot Tub (___)	Stream (___)	Pond (___)
1. No. of times went to	___	___	___	___	___	___
2. Occasions immersed head	___	___	___	___	___	___
3. Occasions swallowed water	___	___	___	___	___	___

V. PRIOR ILLNESS

VI. INCIDENT ILLNESS

Check if Present with day of onset (-3 to +3)

Symptom	Past 3 Days	Next 3 Days
Nausea	___	___
Vomiting	___	___
Diarrhea	___	___
Stomach Ache	___	___
Stomach/Intest. Cramps	___	___
Bloating/Gas	___	___
Anorexia	___	___
Fever	___	___
Headache	___	___
Muscle/Body Aches	___	___
Rhinorrhea	___	___

Column #1 DAY OF ONSET (-3 to +3)			
	Past	Next	
Nausea	___	___	0 1
Vomiting	___	___	0 2
Diarrhea	___	___	0 3
Stomach Ache	___	___	0 4
Stomach/Intest. Cramps	___	___	0 5
Bloating/Gas	___	___	0 6
Anorexia	___	___	0 7
Fever	___	___	0 8
Headache	___	___	0 9
Muscle/Body Aches	___	___	1 0
Rhinorrhea	___	___	1 1

Eye redness, itchy, watery, discharge, pain, or photophobia	____	____	__ __ 1 2
	Check if Present		Coding Column
Symptom	Past 3 Days	Next 3 Days	
Ear ache	____	____	__ __ 1 3
Ear infection	____	____	__ __ 1 4
Skin rash	____	____	__ __ 1 5
Sunburn	____	____	__ __ 1 6
Other	____	____	__ __ 1 7
Confined to bed	____	____	__ __ 1 8
Confined to room	____	____	__ __ 1 9
Saw or called Dr.	____	____	__ __ 2 0

II. INTERVIEWER DATA

1. Interviewer #1 _____ __ __

Date Completed (Julian) _____ _ _/_ _/_ _

Time Completed _____ __ __ __ __

2. Interviewer #2 _____ __ __

Date Completed (Julian) _____ _ _/_ _/_ _

Time Completed _____ __ __ __ __

Appendix B

University of Hawaii at Manoa

Water Resources Research Center
Holmes Hall 283 • 2540 Dole Street
Honolulu, Hawaii 96822

ハワイのビーチに来ていらっしゃる皆さんへ

ハワイ州立大学、及びハワイ州衛生課は現在、ハワイの海岸を使用していらっしゃる皆さんの健康に関する調査を行っています。この調査のために、私どもは海岸ですごされている皆さんの御時間を少し拝借していくつかの簡単な質問に答えて頂いております。もし、調査に御賛同頂けましたら約5分ほどの時間で済みますので、皆さん御自身につきまして、特に最近の健康状態について質問に御答え頂きたいと思います。又、調査の必要上、皆さんに今日の質問に御答え頂いてから3日後にもう一度だけ、同じ様な質問に御答え頂くために私どものほうから皆さんのほうへ御連絡申しあげねばなりません。質問及び、御答え頂いた内容につきましては調査のためのみに用いられ、それ以外の目的で使用されることは一切なく、皆さんのプライバシーに影響を与えるような可能性は全くございませんので御安心ください（2回めの質問後、皆さんの連絡先や名前などについては消去されます）。

皆さんの御協力に心から御礼申し上げます。又、何か御質問などございましたらどうか御気軽にハワイ州衛生課（電話番号586－4337）、又はハワイ州立大学公衆衛生学部のモレン（Morens）博士（電話番号956－8601）まで御連絡ください。ハワイでの御滞在が素晴らしいものになるよう心から願っております。

ハワイ州立大学公衆衛生学部
School of Public Health
University of Hawaii

ハワイ州衛生課
Department of Health
States of Hawaii

University of Hawaii at Manoa

Water Resources Research Center
Holmes Hall 283 • 2540 Dole Street
Honolulu, Hawaii 96822

To Hawaii Beach goers:

The University of Hawaii and the Department of Health are conducting a study on the health of people who use the beaches. We ask a moment of your time to complete a questionnaire. If you agree, we will spend 5 minutes asking you about yourselves and your recent health. We will also need to contact you 3 days later to ask similar questions about your health over the past 3 days. All information will remain confidential and after the second interview, the portion of the files with your name and contact address will be deleted.

We appreciate your help in our research and if you have any questions please contact the Department of Health at 586-4337 or Dr. Morens of the University of Hawaii School of Public Health at 956-8601.